JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. III WASHINGTON, D. C., FEBRUARY 15, 1915 No. 5

LIFE HISTORY OF THE MEDITERRANEAN FRUIT FLY FROM THE STANDPOINT OF PARASITE INTRODUCTION

By E. A. BACK, *Entomological Assistant*, and C. E. PEMBERTON, *Scientific Assistant*, *Mediterranean Fruit-Fly Investigations, Bureau of Entomology*

INTRODUCTION

The ease with which those parasites of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) that are capable of living several months in glass tubes, if receiving intelligent care, can be introduced from western Africa into Hawaii has been most admirably demonstrated by Dr. F. Silvestri, who was engaged by the Hawaiian Board of Agriculture and Forestry to search western Africa for parasites that might be of value in checking the ravages of the fruit fly in Hawaii.[1] While Dr. Silvestri succeeded in introducing parasites at Honolulu, three species of which (*Galesus silvestrii* Kieffer, *Dirhinus giffardii* Silvestri, and *Opius humilis* Silvestri) have since been reared and liberated in large numbers by the Hawaiian Board of Agriculture and Forestry, he failed to introduce *Tetrastichus giffardii*, which, in his opinion, gives greater promise as a parasite in Hawaii than any of the other species introduced. He ascribes his failure to introduce this parasite to its short life and its habit of ovipositing in either the egg or the young larvæ of the fruit fly. On his trip of exploration, which necessitated many stops and side trips, Dr. Silvestri could not be hampered in his movements by such continuous rearings of the host insect as must be undertaken by one carrying to so great a distance this or other short-lived parasites breeding under similar conditions. During the period of a little more than a year in which the writers have been rearing fruit flies in large numbers they have developed certain extremely simple methods for rearing *Ceratitis capitata*. These result in saving much time and in preventing many failures in connection with the effort to introduce parasites of fruit flies from western Africa into the Hawaiian Islands, as they provide a means of keeping on hand the various stages of the fruit fly for the rearing of new generations of parasites.

[1] For a full account of this expedition, see Silvestri, F. Viaggio in Africa per cercare parassiti di mosche dei frutti. 164 p., 69 fig. Portici, 1913. (Bol. Lab. Zool. Gen. e Agr., R. Scuola Sup. Agr. Portici., v. 8, 1914.). For English edition, see Report of an Expedition to Africa in Search of the Natural Enemies of Fruit Flies (Trypaneidae) with Descriptions, Observations, and Biological Notes. 176 p. 24 pl. Honolulu, 1914. (Bul. Hawaii Bd. Agr. and Forestry, no. 3.)

PUPÆ

SECURING MATERIAL

In obtaining a colony of fruit flies the usual method is that of placing infested fruit over sand in some kind of a securely screened container. The writers have found that the easiest method of securing large quantities of pupæ from which to rear adults and pupal parasites is to use any sort of contrivance which will keep the infested fruits free from the sand and at the same time bring the emerging larvæ to a central point, where they may be quickly and easily gathered. If the fruit is allowed to come in contact with the sand, the latter becomes so saturated with the juice of the decaying fruits that it can be freed from the pupæ only with considerable effort and expenditure of time. Plate XLIV, figure 2, represents the first contrivance of this kind used by the writers. It is made of galvanized iron, 36 by 18 inches, with a depth at the lowest point of 24 inches. On the inside, 2 inches from the top, there are narrow supports, which hold in place a tray with handles at both ends and a bottom of galvanized-iron screen with a ¼-inch mesh. The larvæ emerging from the fruit instinctively work their way downward and fall through the screen, and are carried thence by gravity and their own movements through the outlet below into the small container. This arrangement works well with very nearly all the host fruits likely to be used as a source of pupæ, such as *Mumsops elangi*, the rose-apple (*Eugenia jambos*), the kamanis (*Terminalia cattapa* and *Calophyllum inophyllum*), and the strawberry guava (*Psidium cattleyanum*). Such fruits as the mango (*Mangifera indica*) can be used, but they yield so much juice that the inside of the frame becomes so wet that the escaping larvæ often pupate without falling through into the container below, and the sand in the latter becomes more or less saturated.

Previous to the adoption of this method of securing pupæ, there was in general use, both by the Hawaiian Board of Agriculture and Forestry and by the writers, a shallow box about 14 by 12 by 3 inches, ordinarily used by florists. The bottom of this box was covered to a varying depth with sifted sand, on which were placed the infested fruits. During the height of the season of 1913 from five to eight men were employed by the Territorial and Federal authorities in daily removing infested fruits from one box to another containing fresh sand, and in sifting for the pupæ the sand over which the fruit had lain for 24 hours. Besides requiring much time, the prolonged sifting necessary to free the pupæ injured many of them. The writers are now using a frame 6 by 3 feet and 3 feet in depth similar to that shown in Plate XLIV, figure 2. The use of a sufficient number of cheap wooden frames covered with tin well painted will make it possible for one man in several hours to do the daily work formerly requiring six to eight men.

An adaptation of the old box system has been found useful when it has been desirable to keep separate the pupæ from small lots of fruit gathered

from various localitites. One box with a screen bottom of a sufficiently large mesh contains the fruit and is placed over a box of the same size containing sand. By this method the fruit does not need to be handled daily, and the sand below is kept so dry that the larvæ falling through into it are easily sifted (Pl. XLIV, fig. 1).

EMERGENCE OF LARVÆ AND PUPATION

The larvæ leave the fruit in largest numbers at or just after daybreak. Thus, on the 6th of July, 16,624 larvæ emerged between 4 and 9 a. m., as compared with 57 between 9 a. m. and 11 p. m. Siftings made at 6, 7, 8, 9, and 10 a. m., on July 7, yielded 1,006, 448, 171, 95, and 14 larvæ, respectively, during these hourly intervals. On July 8, at 5.20, 6, 7, 8, 9, and 10 a. m., 152, 978, 369, 72, 31, and 14 larvæ, respectively, emerged, as compared with 7 larvæ during the rest of the day. The mean temperature for the period of larval emergence during which these observations were made ranged from 73° to 74° F. Nearly all puparia are formed in from one to two hours during warm weather.

LENGTH OF PUPAL STAGE

From the data included in Table I it will be seen that the minimum length of the pupal stage is 6 days when the mean temperature ranges from about 76° to 79° F. During the warmest Honolulu weather the larger proportion of any lot of pupæ requires from 9 to 11 days before yielding adults. This period may be increased to at least 19 days when the daily means drop to about 69° to 71°.

TABLE I.—*Duration of the pupal stage of the Mediterranean fruit fly*

Date of pupation.	Date of emergence.	Number of adults emerging.	Pupal stage.	Mean temperature.	Date of pupation.	Date of emergence.	Number of adults emerging.	Pupal stage.	Mean temperature.
			Days.	*° F.*				*Days.*	*° F.*
Jan. 16	Feb. 1	1	16	69.6	Apr. 17	Apr. 29	43	12	69.6
Jan. 18	Feb. 3	1	16	70.0	Do....	Apr. 30	1	13	72.7
Jan. 19	Feb. 4	38	16	70.0	Apr. 20	May 1	24	11	73.2
Jan. 20	...do....	18	15	70.0	Do....	May 2	10	12	73.2
Jan. 23	Feb. 5	10	13	70.3	Apr. 22	...do....	1	10	73.4
Jan. 26	Feb. 10	16	15	71.2	Do....	May 3	11	11	73.5
Feb. 13	Feb. 27	130	14	70.8	Apr. 23	May 5	6	12	73.7
Feb. 14	Feb. 28	259	14	70.8	Apr. 24	...do....	9	11	73.9
Feb. 16	Mar. 2	300	14	71.2	Apr. 25	...do....	1	10	73.7
Feb. 18	Mar. 3	300	14	70.3	Do....	May 6	10	11	73.7
Feb. 27	Mar. 13	7	14	70.4	June 4	June 13	1	9	76.1
Do....	Mar. 14	40	15	70.3	Do....	June 14	3	10	76.1
Do....	Mar. 15	15	16	70.4	Do....	June 15	73	11	76.2
Do....	Mar. 17	17	18	70.9	June 8	June 16	2	8	76.2
Do....	Mar. 18	3	19	70.9	Do....	June 17	15	9	76.2
Feb. 28	Mar. 15	88	15	70.5	Do....	June 18	28	10	76.1
Do....	Mar. 16	56	16	70.9	Do....	June 19	53	11	76.1
Do....	Mar. 17	14	17	71.0	Do....	June 20	33	12	76.1
Mar. 1	Mar. 16	29	15	70.2	Do....	June 21	5	13	76.1
Do....	Mar. 17	9	16	71.1	June 10	June 10	5	0	76.4
Mar. 14	Mar. 26	1	12	73.9	Do....	June 19	14	9	76.3
Do....	Mar. 27	1	13	73.7	Do....	June 20	101	10	76.3
Apr. 11	Apr. 22	4	11	71.2	Do....	June 21	160	11	76.2
Do....	Apr. 23	8	12	71.4	Do....	June 22	7	12	76.1
Do....	Apr. 24	3	13	71.6	Do....	June 23	3	13	76.3
Apr. 13	...do....	6	11	72.0	July 13	July 20	1	7	79.2
Do....	Apr. 25	4	12	72.9	Do....	July 21	3	8	79.2
Apr. 15	Apr. 26	2	11	72.4	Do....	July 22	39	9	79.2
Do....	Apr. 27	3	12	72.5	Do....	July 23	43	10	79.1
Apr. 17	Apr. 28	15	11	72.6	Do....	July 24	3	11	79.0

It was found that in Bermuda, when the monthly means range from 62.5° to 64.8° F., the pupal stage was lengthened to about 31 days under normal conditions. The writers have found that the Mediterranean fruit fly can pass from egg to adult if kept in the dark in cold storage at 56° to 57° and that at this temperature practically all pupæ yield adults from 37 to 41 days after pupation. Pupæ placed in cold storage in the light at a temperature varying between 58° and 62° were apparently unaffected by the cold, except that the length of the stage was increased to from 29 to 31 days for pupæ which were about 3 hours old when placed in cold storage. In carrying pupæ from place to place for rearing purposes a temperature of less than 56° to 60° is not advised, as great mortality occurs. Thus, from about 300 pupæ 1 day old placed in cold storage at about 50° on June 2 and removed to a normal summer temperature at Honolulu on July 22 only 8 adults emerged during the period from July 24 to 26.

ADULTS

The adults of the Mediterranean fruit fly emerge in largest numbers early in the morning during warm weather and more scatteringly during cool weather.

CARE OF ADULTS

As adults die in greatest numbers within 48 hours after emergence, or within 72 hours at the longest, if food is not given them, those required for future observations must be transferred to a place where they may be fed and cared for daily. In this work the writers have found glass jars 9 by 12½ inches covered with cheesecloth very convenient. Such jars will hold from 200 to 300 flies in good condition. Fruit juices of almost any sort are eagerly eaten. Water slightly sweetened with pineapple (*Ananas ananas*) syrup was used with good results by the writers for many months, but was replaced later by a mixture of water and finely divided parts of papaya (*Carica papaya*). When fed with such diluted food, adults thrive best on two feedings a day, one in the morning and one late in the afternoon. The food may be applied in finely divided drops to the sides of the jar by flirting the mixture forcibly against the cheesecloth covering by means of a snapping movement of the thumb and forefinger. The adults feed greedily and soon become distended. In this condition many fall and rest upon the bottom of the jar; hence, the less food falling on this portion of the jar the fewer will be the deaths resulting from entanglement in it. If the flies are not required for oviposition, they can be kept alive far more easily by suspending within the jar a juicy fruit upon which they may feed. One mango, the skin of which had been broken in numerous places, served to keep alive from 100 to 200 flies for one week. Feeding by flirting mixtures through the cloth covering causes the sides of the jar to become soiled quickly and necessitates

the changing of adults to clean jars every two or three days. When fed with suspended fruit, the flies require no changing for at least two weeks. Mortality among the flies increases rapidly in badly soiled jars.

LENGTH OF LIFE

Well-fed Mediterranean fruit flies have been kept alive in these jars more than five months. One fly that emerged on December 31, 1913, lived until May 11, 1914, or 131 days. Other flies, which emerged on February 28, 1914, are alive at the date of this writing—August 1. Usually about 50 per cent of the flies may be expected to die during the first two months after emergence. When the monthly mean temperature averages about 76° to 79° F., comparatively few flies live to be more than 3 months old. When kept at a temperature ranging from 58° to 63°, the writers believe from accumulating data, that especially strong adults will live to be more than 6 months old.

SEXUAL MATURITY

Neither male nor female flies are sexually mature when they emerge from the pupa. Males show sexual activity often four days after emergence, and copulation has been observed five days after emergence. When the daily mean temperature averages from 76° to 78° F., the larger percentage of females is ready to mate from six to eight days after eclosion. Adults that emerged on May 23 and 24 and were placed on May 25 in the light in cold storage at 61° to 64° were not observed to mate until June 5, when 14 days old. Copulation may occur at any time throughout the day.

OVIPOSITION

Oviposition may take place in Hawaii as early as 5 days after emergence during very warm weather, but not for about 10 days when the temperature ranges between 68° and 72° F. At mean temperatures above 74° various lots of adults will yield large numbers of eggs from 7 to 8 days after emergence. Adults oviposit best at temperatures varying from 70° upward, but have been observed depositing eggs at 65° to 67°, and in cold storage in the light at about 62°.

It is impossible at this writing (August 1, 1914) to state the full capacity for egg deposition possessed by females of this species. The number of eggs found at any time in the reproductive organs is no indication of the total number of eggs an individual female is capable of depositing, for new eggs are being formed continually throughout life. The data in Table II show that during the first 18 weeks of her life one adult deposited 499 eggs and was still in a thrifty condition. Two other females during the same time deposited 416 and 336 eggs, respectively. A fourth female living but 80 days deposited 312 eggs. Usually females die soon after

they cease to oviposit. Fly No. 9 in Table II is an exception, as she deposited but 3 eggs in one puncture during her life of 68 days and lived without ovipositing for 35 days before she died. The data in Table II give the capacity for oviposition possessed by females up to 18 weeks of age. Those in Table III show that this capacity is fairly well maintained by certain females at least during the fifth month after emergence. Thus fly No. 5 of Table II deposited on an average 4.5 eggs per day after she began ovipositing, while fly No. 1 of Table III deposited an average of 4.6 eggs per day for the first 24 days of the fifth month of her life.

TABLE II.—*Daily rate of oviposition of the Mediterranean fruit fly. Females emerged on April 4, 1914, and were placed with fruit on April 14, 1914*

Date of oviposition.[a]	Number of eggs deposited.								
	Fly No. 1.	Fly No. 2.	Fly No. 3.	Fly No. 4.	Fly No. 5.	Fly No. 6.	Fly No. 7.	Fly No. 8.	Fly No. 9.
Apr. 16	3	0	0	0	0	0	0	0	0
17 to 20	14	7	11	24	19	14	0	0	0
20 to 22	0	0	0	0	0	0	5	0	0
22 to 25	0	0	7	0	0	0	0	2	0
25 to 27	0	7	13	0	13	0	14	0	0
27 to 29	11	8	6	0	16	15	0	20	0
29 to 30	0	0	0	0	17	16	23	0	0
May 1 to 3	0	0	0	3	25	19	19	13	0
3 to 5	25	0	0	0	12	0	0	0	3
6	2	0	0	0	0	0	9	0	0
7	8	0	0	0	19	12	2	8	0
8	9	0	0	0	9	3	1	0	0
9	9	0	0	0	7	7	4	3	0
10	0	0	0	2	0	6	2	0	0
11	17	0	0	0	7	3	0	0	0
12	4	4	0	0	10	4	8	0	0
13	14	0	0	0	7	8	11	0	0
14	8	4	0	0	10	2	9	0	0
15	8	0	0	5	5	6	6	1	0
16	5	0	0	0	11	6	8	14	0
17	8	0	0	0	0	4	3	0	0
18	2	0	0	13	3	3	3	8	0
19	3	4	0	0	5	0	3	9	0
20	2	(b)	0	0	6	0	3	11	0
21	0	.....	0	0	2	2	9	8	0
22	5	.....	0	0	5	0	1	9	0
23	10	.....	20	4	6	10	4	4	0
24	4	.....	2	18	3	1	4	7	0
25	5	.....	19	4	2	3	3	3	0
26	0	.....	2	9	6	2	0	3	0
27	0	.....	6	15	9	0	5	8	0
28	0	.....	(c)	3	3	5	3	4	0
29	9	.....	.....	9	3	14	7	7	0
30	1	.....	.....	12	0	8	10	4	0
31	0	.....	.....	9	6	13	21	8	0
June 1	0	.....	.....	8	3	10	5	5	0
2	0	.....	.....	13	5	6	14	0	0
3	0	.....	.....	9	12	4	7	2	0

[a] Dates on which none of the flies oviposited are omitted from the table.
[b] Died on this date.
[c] Escaped on this date.

TABLE II.—*Daily rate of oviposition of the Mediterranean fruit fly. Females emerged on April 4, 1914, and were placed with fruit on April 14, 1914*—Continued

Date of oviposition.	Number of eggs deposited.								
	Fly No. 1.	Fly No. 2.	Fly No. 3.	Fly No. 4.	Fly No. 5.	Fly No. 6.	Fly No. 7.	Fly No. 8.	Fly No. 9.
June 4	5	.....	.....	3	3	4	3	9	0
5	0	.....	.....	7	8	0	6	5	0
6	0	.....	.....	4	6	0	4	3	0
7	0	.....	.....	18	11	15	15	12	0
8	0	.....	.....	0	0	1	0	1	0
9	0	.....	.....	15	7	8	12	9	0
10	0	.....	.....	6	5	11	1	2	(a)
11	0	.....	.....	12	7	11	9	6	.....
12	(a)	.....	.....	0	6	3	3	6	.....
13	.....	.....	.....	7	2	4	7	0	.....
14	.....	.....	.....	4	2	7	1	8	.....
15	.....	.....	.....	0	7	2	7	2	.....
16	.....	.....	.....	0	9	4	5	3	.....
17	.....	.....	.....	0	6	5	0	2	.....
18	.....	.....	.....	0	0	0	0	3	.....
19	.....	.....	.....	0	6	3	3	8	.....
20	.....	.....	.....	4	4	12	0	4	.....
21	.....	.....	.....	10	10	7	0	9	.....
22	.....	.....	.....	4	0	0	0	5	.....
23	.....	.....	.....	10	2	(a)	9	0	.....
24	.....	.....	.....	0	9	.....	12	12	.....
25	.....	.....	.....	0	7	.....	3	3	.....
26	.....	.....	.....	0	9	.....	0	2	.....
27	.....	.....	.....	5	6	.....	7	4	.....
28	.....	.....	.....	16	14	.....	12	4	.....
29	.....	.....	.....	6	5	.....	0	2	.....
30	.....	.....	.....	0	2	.....	6	2	.....
July 1	.....	.....	.....	3	7	.....	2	8	.....
2	.....	.....	.....	10	0	.....	7	1	.....
3	.....	.....	.....	3	0	.....	3	1	.....
4	.....	.....	.....	7	4	.....	8	6	.....
5	.....	.....	.....	0	6	.....	2	3	.....
6	.....	.....	.....	0	3	.....	4	0	.....
7	.....	.....	.....	0	2	.....	0	0	.....
8	.....	.....	.....	(a)	3	.....	9	2	.....
9	.....	.....	.....	.....	6	.....	3	4	.....
10	.....	.....	.....	.....	2	.....	3	4	.....
11	.....	.....	.....	.....	0	.....	8	2	.....
12	.....	.....	.....	.....	9	.....	4	0	.....
13	.....	.....	.....	.....	2	.....	0	0	.....
14	.....	.....	.....	.....	5	.....	3	5	.....
15	.....	.....	.....	.....	2	.....	0	3	.....
17	.....	.....	.....	.....	4	.....	0	0	.....
19	.....	.....	.....	.....	0	.....	0	4	.....
20	.....	.....	.....	.....	0	.....	2	0	.....
22	.....	.....	.....	.....	14	.....	0	0	.....
23	.....	.....	.....	.....	0	.....	3	0	.....
24	.....	.....	.....	.....	11	.....	4	0	.....
Total	191	34	86	314	[b]499	312	[b]416	[b]336	3

[a] Died on this date.
[b] Aug. 1, 1914: These females are still alive and promise to oviposit for some time yet

TABLE III.—*Daily rate of oviposition of the Mediterranean fruit fly. Females emerged on February 28, 1914; hence, were 4 months old on June 28, 1914*

Date of oviposition.[a]	Number of eggs deposited.					
	Fly No. 1.	Fly No. 2.	Fly No. 3.	Fly No. 4.	Fly No. 5.	Fly No. 6.
July 1	0	0	0	0	6	0
2	9	0	0	0	2	0
3	0	0	0	0	4	0
4	10	4	0	0	0	6
5	4	0	0	0	0	0
6	5	6	0	4	0	0
7	0	0	0	0	0	12
8	10	6	0	0	0	6
9	0	4	0	9	0	13
10	15	6	0	3	0	2
11	5	2	0	0	0	4
13	12	7	(b)	5	0	0
14	2	0	.....	5	0	(b)
15	7	9	.....	0	0	.....
16	0	8	.....	0	0	.....
17	13	3	.....	11	0	.....
18	5	0	.....	4	0	.....
19	6	2	.....	0	0	.....
20	0	5	.....	4	0	.....
21	5	2	.....	6	0	.....
22	0	8	.....	7	0	.....
23	0	2	.....	0	0	.....
24	2	6	.....	13	(b)	.....
Total	[c] 110	[c] 80	0	[c] 71	12	43

[a] Dates on which none of the flies oviposited are omitted from the table.
[b] Died on this date.
[c] Aug. 1, 1914: These females are still alive and give promise of sexual activity for some time to come.

Oviposition experiments are still in progress; hence, no estimate can be made of the maximum egg-laying capacity of the female *Ceratitis capitata*. It is evident, however, from the data in Tables II and III that the females lay small batches of eggs quite regularly throughout life.

DIFFERENCES IN HABIT BETWEEN THE ADULT MEDITERRANEAN FRUIT FLY AND THE ADULT MELON FLY

Those desiring to rear the melon fly (*Bactrocera cucurbitae* Coq.) or parasites of its eggs and young larvæ will find marked differences in habit between this and *Ceratitis capitata*. These same differences will probably be found to occur between species of Dacus and Ceratitis. There is very little difference found by the writers in the egg, larval, and pupal stages. The adults of the melon fly are far more hardy than adults of *C. capitata*. The writers have on hand many adults 6 months old which give every promise of living indefinitely, as very few have died during the last few months and those living are as active as when newly emerged. The adult melon fly exhibits no sexual activity for such a long period

after emergence that one is likely to be discouraged in obtaining eggs. While the sexes of the Mediterranean fruit fly are sexually active throughout the day, the melon flies become active only at sunset. From sunset until dark copulation occurs and lasts in many instances until daybreak, inasmuch as numerous pairs have been observed in coition at midnight and at dawn, when all flies are very quiet. Adults issuing from pupæ on May 24 did not mate until June 13, or 20 days after emergence, although they were observed every evening. The majority of females in this lot did not mate until 25 days old. The daily mean temperatures for the period from May 24 to June 13 averaged 75.5° F.

The female *Bactrocera cucurbitae* is more irregular in her habits of oviposition. As shown by the data in Table IV, she lays more consistently a large number of eggs at one time.

TABLE IV.—*Daily rate of oviposition of the melon fly (Bactrocera cucurbitae). Emerged on May 25 and placed separately with fruit on June 25, 1914*[a]

Date of oviposition.[b]	Number of eggs deposited.						
	Fly No. 1.	Fly No. 2.	Fly No. 3.	Fly No. 4.	Fly No. 5.	Fly No. 6.	Fly No. 7.
July 10	0	0	0	0	0	23	0
11	0	0	13	0	17	0	0
15	14	0	0	0	0	12	0
17	0	0	9	0	14	0	0
18	19	0	0	0	0	0	0
19	0	0	0	0	0	0	19
21	0	0	0	0	0	6	0
22	13	0	0	0	0	0	0
23	0	0	0	0	10	0	0
24	0	0	0	3	0	0	0
26	0	0	0	0	0	23	0
27	29	0	0	0	0	0	0

[a] These 7 females were all alive on July 27.
[b] Dates on which none of the flies oviposited are omitted from the table.

The data in Table IV were secured from young females during the early period of sexual activity. Other data on file show that females over 5 months old deposit quite as freely.

EGGS

Eggs may be obtained most easily for experimental work by suspending fruit on a string in a jar containing adults, after the latter have begun to mate. In Plate XLV, figure 1, is illustrated this simple method of obtaining eggs during a known period. If the epidermis of the fruit is shaved off in several places oviposition will be made easier. The removing of eggs either from the body of the female or from the sides of the containing jar, as practiced by several workers, has not given good results.

If it is desired to keep constant watch over eggs they may be dissected easily from the egg cavity and spread upon a section cut from any firm

leaf. The leaf should then be inserted into a small vial, an absorbent-cotton plug added to force the leaf well toward the bottom, and the vial with contents inverted and partially submerged in a jar of water. It has been found by checks that eggs, when handled in this manner, develop normally unless injured in the transfer. An ordinary moist chamber does not seem to serve the purpose so well.

TABLE V.—*Duration of the egg stage of the Mediterranean fruit fly*

Number of eggs under observation.	Eggs deposited.	Eggs hatched.	Average mean temperature
			°F.
88	Jan. 21–22, 4 p. m. to 10 a. m.	Jan. 26, 6 a. m. to 3 p. m.	68.7
350	Mar. 9	Mar. 12–13, 4.30 p. m. to 8 a. m.	70.2
264	Mar. 22–23, 2 p. m. to 9 a. m.	Mar. 26, a. m.	71.0
135	Do.	Mar. 27, a. m.	71.0
236	Do.	Mar. 28–29, 8 a. m. to 6 a. m.	71.3
12	Do.	Mar. 29–30, 6 a. m. to 10 a. m.	71.0
102	Mar. 27, 9 a. m. to 1 p. m.	Mar. 30, a. m.	71.0
695	Do.	Mar. 30, a. m., to Mar. 31, a. m.	71.0
28	Do.	Mar. 31–Apr. 1, 9 a. m. to 8 a. m.	71.0
3	Do.	Apr. 1–2, 9 a. m. to 8 a. m.	71.0
50	Mar. 27, a. m.	Mar. 31, a. m.	71.0
102	May 19, 3 p. m. to 6 p. m.	May 22, 7 a. m. to 10 a. m.	76.0
176	May 12–13, 3 p. m. to 12 m.	May 15, a. m.	75.0
243	Do.	May 15–16, 2 p. m. to 8 a. m.	75.0
13	June 17–18, 4 p. m. to 8 a. m.	June 20, 11 a. m.	77.0
2	Do.	June 19–20, 6 p. m. to 8 a. m.	77.3
44	June 18, 1.30 p.m. to 3.30 p. m.	June 20–21, 6 p. m. to 8 a. m.	77.0
90	June 19, 10 a. m. to 1 p. m.	June 21–22, 6 p. m. to 8 a. m.	76.6
72	June 19–20, 4 p.m. to 8.30 a.m.	June 21–22, 6 p. m. to 9 a. m.	77.0
77	June 20, 9 a. m. to 4 p. m.	June 22–23, 6 p. m. to 7.30 a. m.	77.0
60	June 23, 10 a. m. to 4 p. m.	June 25–26, 6 p. m. to 6 a. m.	76.8
12	Do.	June 26, 7 a. m. to 11.45 a. m.	76.8
63	June 24, 1.30 p.m. to 4.30 p.m.	June 26–27, 4.30 p. m. to 6 a. m.	77.0
24	July 1, 1.30 p. m. to 5 p. m.	July 3–4, 5 p. m. to 6.30 a. m.	77.0
134	July 15, 3.30 p. m. to 4.30 p.m.	July 17, 4.30 p. m. to 6 p. m.	78.9
128	Do.	July 17, 6 p. m. to 8 p. m.	78.9
20	Do.	July 17, 9 p. m. to 10 p. m.	78.9
12	Do.	July 18, 10 a. m to 4 p. m.	78.3
74	July 15–16, 12 m. to 5 p. m.	July 18	80.0
18	Do.	July 19, a. m.	79.8
2	Do.	July 19, 9 a. m. to 1 p. m.	79.8
2	Do.	July 20, p. m.	79.5
101	Nov. 13–14, 4 p. m. to 9 a. m.	Nov. 16, 2 a. m. to 6 a. m.	75.5
10	Do.	Nov. 16, 6 a. m. to 11 a. m.	75.5
3,442			

Plate XLV, figure 2, is reproduced from a photograph of an apple that had been hung in a jar with flies for one day. Each dark spot represents a puncture. The entire apple was estimated to contain over 2,000 eggs. As the females live over long periods and oviposit freely throughout life eggs may easily be obtained daily for parasitic work while experimenters are en route from one country to another. Apples are

probably the most satisfactory fruit for egg deposition during a voyage, as they may be had at almost all points and they keep for a considerable length of time. While the female shows decided preference for certain fruits, she will oviposit, when forced, in almost any fruit if oviposition is not prevented by physical conditions. Fruit in a hard and semiripe condition is better for oviposition than fully ripe fruit, as the latter is likely to be more juicy, and very juicy fruits often cause a high mortality among eggs and young larvæ.

During very warm weather eggs hatch in about two days. It will be seen, however, from the data in Table V that the length of the egg stage is considerably increased by lower temperatures.

At a mean temperature of 78.9° F. 134 eggs hatched between 49 and 50 hours after being deposited, although 12 eggs deposited at the same time did not hatch until from 66 to 72 hours. At a mean temperature of 71° F. 695 eggs hatched within 72 hours, while 3 hatched in from 120 to 144 hours, or about 6 days after deposition. Eight eggs hatched between 4 and 4½ days after deposition at a mean temperature of 68.7°. At 59° to 62° eggs hatched in cold storage in from 5 to 7 days, and at 54° to 57° in from 7 to 14 days after deposition.

LARVÆ

The larvæ pass through three instars, which may be readily distinguished. Of chief interest in connection with this paper is the length of larval life. The data in Table VI show that this may be as short as 5 or 6 days when the mean temperatures average about 77° F. One larva at this temperature required 14 days to become full grown.

The character of the fruit often influences the length of the larval stage. In citrous fruits, especially in limes and lemons, it appears to be longer. Thus larvæ require 14 to 26 days to reach maturity in a ripe lemon, as compared with 10 to 15 days in a green peach. Citrous fruits, however, are not desirable for rearing work with either flies or their egg parasites. For successful rearing work, where it is desired to prolong the length of larval life by slightly lowered temperatures, adults should not be permitted to lay more than 50 to 150 eggs in such fruits as the apple. The feeding of larvæ in overinfested fruits brings about such a rapid decay that few become well grown. At 56° to 57° F. larvæ have become full grown and emerged from slightly infested apples in a refrigerator over a period ranging from 36 to 53 days after hatching.

TABLE VI.—*Duration of the larval stage of the Mediterranean fruit fly*

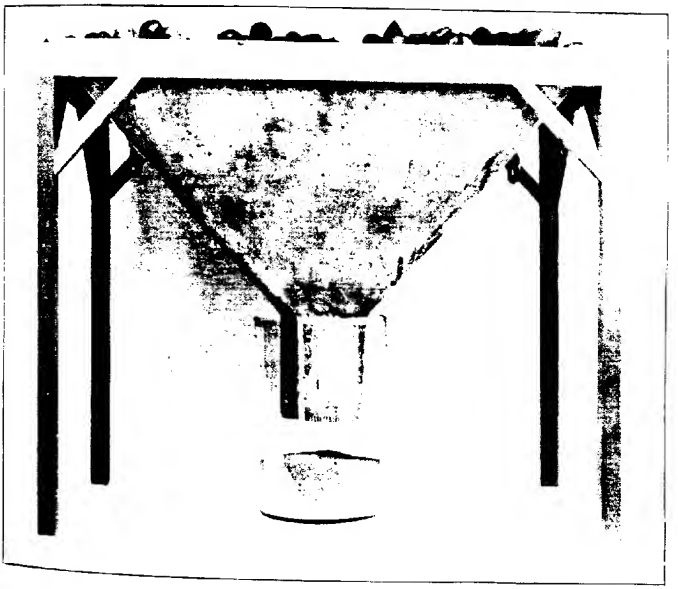
Number of specimens under observation.	Approximate period of development.	Host fruit.	Instar 1.	Instar 2.	Instar 3.	Larval stage.	Mean temperature for period of development.
			Hrs.	*Hrs.*	*Hrs.*	*Days.*	°*F.*
1	June 12 to 18	Papaya	38	36	48	5.1	77.6
2	Do	do	36	48	48	5.5	77.6
1	Do	do	36	48	48	5.5	77.6
1	Do	do	48	48	48	6.0	77.6
1	June 19 to 25	do	26	30	72	5.3	76.4
1	Do	do	48	24	72	6.0	76.4
12	June 19 to 26	do	48	24	96	7.0	76.6
1	June 19 to 27	do	48	30	96	7.2	76.6
1	June 19 to July 1	do	48	24	216	12.0	77.0
1	June 19 to July 3	do	48	27.5	264	14+	77.1
2	June 22 to July 2	Green peach				10	77.2
7	June 22 to July 3	do				11	77.2
6	June 26 to July 6	Hard peach				9.5	77.4
4	June 26 to July 2	Ripe peach				6	77.8
3	June 26 to July 3	do				7	77.7
18	Mar. 31 to Apr. 10	Green peach				10	69.6
12	Mar. 31 to Apr. 11	do				11	69.8
3	Mar. 31 to Apr. 12	do				12	70.0
1	Mar. 31 to Apr. 13	do				13	70.3
1	Mar. 31 to Apr. 15	do				15	71.0
12	Mar. 13 to 27	California lemon				14	70.2
17	Mar. 13 to 28	do				15	70.3
14	Mar. 13 to 29	do				16	70.3
20	Mar. 13 to 30	do				17	70.4
8	Mar. 13 to 31	do				18	70.4
3	Mar. 13 to Apr. 1	do				19	70.5
3	Mar. 13 to Apr. 2	do				20	70.5
2	Mar. 13 to Apr. 3	do				21	70.4
1	Mar. 13 to Apr. 4	do				22	70.4
1	Mar. 13 to Apr. 8	do				26	70.4

PLATE XLIV

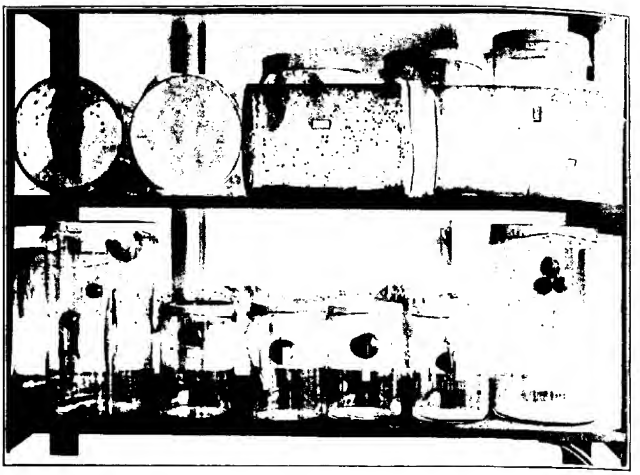
Fig. 1.—Wooden boxes, 14 by 12 by 3 inches in size, used in obtaining pupæ of fruit flies. The upper box, with a coarse screen bottom, contains the infested fruit; from which the larvæ drop through the screen into the lower box containing dry sand. Original.

Fig. 2.—Contrivance used for keeping the infested fruit free from the sand and bringing the emerging larvæ to a central container where they may be gathered quickly. Original.

1



2



1

2

PLATE XLV

Fig. 1.—Method of keeping adult fruit flies alive over long periods. The jars in the lower row contain suspended fruits in which the females readily oviposit. Original.

Fig. 2.—An apple after having been suspended for one day in a jar containing Mediterranean fruit flies. Each dark spot represents a puncture containing from 1 to 30 eggs. The apple is too heavily infested for practical work in rearing parasites. Original.

RELATION OF SIMULTANEOUS OVULATION TO THE PRODUCTION OF DOUBLE-YOLKED EGGS[1]

By MAYNIE R. CURTIS,
Assistant Biologist, Maine Agricultural Experiment Station

INTRODUCTION

In an earlier paper[2] it was shown that double-yolked eggs differ greatly in the number of the normal egg envelopes common to the two yolks. The explanation was then offered that two eggs may come together at any level of the oviduct. If the union is anterior to the isthmus ring, a double-yolked egg results. It was also pointed out that while some of the doubling of eggs is no doubt due to the delay or the backward movement of the first egg, nevertheless an unusually rapid succession of ovulations is necessary to account for the occurrence of double-yolked eggs within a clutch.

The purpose of this paper is, first, to present further data regarding the structural variations in double-yolked eggs and the relation of these variations to the functional divisions of the oviduct; and, second, to record observations bearing upon the relation between simultaneous ovulations and double-yolked egg production.

CLASSIFICATION OF DOUBLE-YOLKED EGGS BASED ON THE NUMBER OF COMMON EGG ENVELOPES

In the earlier paper[3] it was shown that yolks with separate vitelline membranes may have a complete set of common egg envelopes, including common chalazal membranes—"chalaziferous layers" (Pls. XLVI, fig. 3, and XLVII, fig. 1); or they may have separate chalazal membranes, all their other envelopes being common (Pl. XLVII, fig. 2); or they may have some separate and some common thick albumen layers (Pl. XLVIII, fig. 1); or finally they may have entirely separate thick albumen layers but common egg membrane and shell (Pl. XLVIII, fig. 2). A large series of double-yolked eggs shows every possible stage, from cases where the two yolks are flattened together so tightly within the common chalazal membrane that they resemble a single large yolk to eggs in which the doubleness is visible externally by a depressed ring around the shell (Pl. XLIX, fig. 1). In such cases there is often a thin fold of membrane

[1] Studies on the Physiology of Reproduction in the Domestic Fowl.—XI. This paper is the eleventh in a series published in various biological journals and agricultural experiment station bulletins.
Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 75.

[2] Curtis, M. R. Studies on the physiology of reproduction in the domestic fowl.—VI. Double- and triple-yolked eggs. *In* Biol. Bul., v. 26 no. 2, p. 55-83. 1914.

[3] Curtis, M. R. Op. cit.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. III, No. 5
Feb. 15, 1915
Maine—1

75012°—15——2

projecting into the albumen at the deepest part of the furrow (Pl. XLIX, fig. 2). Although the eggs form a continuous series, they can be separated with reasonable accuracy into three classes or types:

Type I.—Double-yolked eggs having the entire set of egg envelopes common to the two yolks.

Type II.—Double-yolked eggs having all or part of the thick albumen common to the two yolks, but with separate chalazal membranes.

Type III.—Double-yolked eggs in which the yolks have entirely separate thick albumen envelopes but a common egg membrane and shell.

RELATION OF THE NATURE OF THE DOUBLING OF THE EGG TO THE SEVERAL FUNCTIONAL DIVISIONS OF THE OVIDUCT

The most obvious interpretation of the various types of doubling observed in double-yolked eggs is that the two components may come together at any level of the oviduct from the ostium to the isthmus ring. The eggs classified as type I arise when the union of the two yolks occurs in that part of the duct where chalazal membrane is secreted (probably the funnel and funnel neck); type II, when the union is at any level in the albumen-secreting region more than the length of the first egg anterior to the isthmus ring; and type III, when the union occurs while the first egg is passing the isthmus ring.

Observations of the relation of the egg envelopes were made on nearly every double-yolked egg produced at the plant of the Maine Experiment Station during the past year. Data were collected on 131 eggs with two normal yolks and a common egg membrane and a shell. Only 21, or 16.03 per cent, were united in a common chalazal membrane, showing that they had passed practically the entire length of the oviduct together. In contrast to this, 93, or 70.99 per cent, had separate chalazal membranes but with all or part of their thick albumen common to the two yolks. These eggs showed every possible gradation from two yolks with entirely common thick albumen envelopes to cases where the two component eggs were contained in a very thin layer of common thick albumen. They had apparently come together at every possible level of the duct from a point in the funnel where the chalazal membranes are complete to a point near the lower end of the albumen-secreting region. Seventeen eggs, or 12.97 per cent, had entirely separate thick albumen envelopes. They had evidently come together while the first component was passing the isthmus ring.

The large proportion of double-yolked eggs with all or part of their thick albumen common to the two yolks is easily explained by the length of the portion of the duct in which they may come together. A photograph of the oviduct opened out to show the various regions is reproduced in Plate I. There is no visible line of demarcation between the funnel

(*A*), where the chalazal membrane and chalazas are probably secreted, and the albumen-secreting portion (*B*). There is, however, a difference in the general appearance of the glandular ridges and the microscopic character of the glands for a distance of 5 to 8 cm. in an adult laying barred plymouth rock fowl. There is a definite line of demarcation, the isthmus ring (*x*), where the egg membrane begins to be secreted. The length of the duct from the mouth of the funnel to the isthmus ring has been determined for 57 normal laying barred plymouth rock fowls. The mean length was 46.3 cm.

The portion of the oviduct in which two yolks can unite and have all the egg envelopes common is probably within the length of the funnel—that is, from 5 to 8 cm. The portion where they will have all their envelopes separate, except the egg membrane and shell, probably roughly approximates the length of the first egg when passing into the isthmus, or from 5 to 7 cm. The union of two eggs in any other part of the duct from the ostium to the isthmus ring (30 to 35 cm.) would result in the formation of a double-yolked egg with all or part of the thick albumen common to both yolks. If we take as the means of the above figures 7, 33, and 6 cm. and calculate the percentage that each is of the total length from ostium to isthmus ring we shall have the expected percentage of double-yolked eggs of each type, if the probability of the union of two yolks is equal at every level of the duct. The number and percentage of eggs of each type observed and the number and percentage expected on the above assumption are given in Table I.

TABLE I.—*Number and percentage observed of each type of double-yolked eggs and the number and percentage expected, if there is an equal probability of the union of the two components at every level of the oviduct*

Type.	Number.		Percentage.	
	Observed.	Expected.	Observed.	Expected.
I (all egg envelopes common)	21	19.94	16.03	15.22
II (separate chalazal membranes; all or part of thick albumen common)	93	93.98	70.99	71.74
III (separate thick albumen envelopes; common membrane and shell)	17	17.08	12.98	13.04
Total	131	131.00	100.00	100.00

The close agreement between the data for the eggs observed and those expected in each type supports the conclusion that the union of the component eggs occurs indiscriminately at all levels of the oviduct. While the eggs observed formed a graded series and the divisions of the duct are somewhat rough, nevertheless, variations in classification within any possible range of observation could not reverse the conclusion that the short distances of duct which could yield eggs of the first and

third type and the comparatively long portion where the union of two eggs could result in an egg of the second type are obviously in accord with the observed percentage of the eggs of each type.

THE QUESTION OF THE SIMULTANEOUS OVULATION OF THE TWO YOLKS OF A DOUBLE-YOLKED EGG

On purely theoretical grounds Parker [1] explains the origin of double-yolked eggs as the "simultaneous or almost simultaneous" discharge of two yolks from the same or separate follicles. He suggests that probably when the two yolks are inclosed in the same vitelline membrane they come from the same follicle, but that when they are in separate membranes they are probably from separate follicles.

Glaser [2] suggests that it is not necessary to assume simultaneous ovulation in the case of the two yolks of a double-yolked egg, since the first yolk may remain in the infundibulum until the next normal ovulation. The present author [3] has further suggested, first, that this delay of the first egg may occur at any level of the duct anterior to the isthmus ring; second, that the first egg may be moved back up the duct by antiperistalsis; and, third, that a yolk ovulated into the body cavity may be later picked up by the funnel and inclosed with its successor in a double-yolked egg.

It is probable that double-yolked eggs arise from some or all of these causes. However, as was pointed out in the previous paper,[3] at least an abnormally close succession of ovulations is necessary to account for a daily succession of double-yolked eggs or of double-yolked eggs laid after a series of normal daily eggs.

No bird belonging to the flock of the Maine Experiment Station during the last six years has produced double-yolked eggs on successive days, but a large number of fowls have produced double-yolked eggs within a series of normal daily eggs. For example, fowl No. 2K produced a double-yolked egg as the seventh egg of a 10-egg clutch, No. 37M one as the sixth egg of a 13-egg clutch, and No. 306K produced double-yolked eggs both as the second and fourth eggs of the same 6-egg clutch. In fact, in 43, or 36.44 per cent, of the 118 cases on which we have complete data the bird which produced a double-yolked egg had laid a normal egg on the preceding day. In these cases it seems certain that the period between ovulations must have been much shorter than the normal period. Further, in 17, or 14.40 per cent, of the 118 cases the bird laid normal eggs on both the preceding and following days. In these cases the evidence for a heightened rate of fecundity is unmistakable, although the ovulations which furnished the yolks for the double-yolked egg may not have been simultaneous.

[1] Parker, G. H. Double hens' eggs. *In* Amer. Nat., v. 40, no. 469, p. 13–25, 1 fig. 1906. Bibliography, p. 23–25.

[2] Glaser, Otto. The origin of double-yolked eggs. *In* Biol. Bul., v. 24, no. 3, p. 175–186. 1913.

[3] Curtis, M. R. Op. cit.

POSSIBILITY OF A RELATION BETWEEN THE RATE OF FECUNDITY AND THE TYPE OF DOUBLING OF THE DOUBLE-YOLKED EGG

It has been shown in previous paragraphs, first, that one-sixth of all the double-yolked eggs show by their structure that the two yolks have passed practically the entire length of the oviduct together (16 per cent have the complete set of egg envelopes common to the two yolks); and, second, that in more than one-third (36.44 per cent) of the cases of double-yolked eggs the two yolks must have been ovulated at an abnormally short interval, since these double-yolked eggs were laid on days following the production of a normal egg. Or, to put the matter in another way, in 43 out of 118 cases the two yolks must have been ovulated in less than the normal time, while in only 19 cases did the two yolks pass the entire length of the oviduct together. The analysis may be carried still farther: Of the 43 cases in which an egg had been laid on the preceding day only 7, or 16.28 per cent, were eggs with the two yolks inclosed in common chalazal membranes—that is, even where we have evidence from the egg record of the fowl that the ovulations must have been unusually rapid the structure of the egg indicates that they were simultaneous in only a small percentage of the cases. This is shown in the first column of Table II. This column also shows that in a few of these cases the entire thick albumen envelopes of the two yolks are separate. This indicates that the two eggs have not united until very near the end of the albumen-secreting portion of the oviduct.

TABLE II.—*Number and percentage of each type of double-yolked eggs occurring as single eggs, or preceded or followed within one day by a normal egg*

Type.	Cases in which a double-yolked egg occurred on the day following a normal egg.		Cases in which a normal egg was laid on the day following, but not on the day preceding the double-yolked egg.		Cases in which a double-yolked egg occurred as a single egg.		Double-yolked eggs observed.	Percentage of total.
	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Total number.	
I (all egg envelopes common)	7	36.84	6	31.58	6	31.58	19	16.10
II (all or part of thick albumen common)	33	39.76	20	24.10	30	36.14	83	70.34
III (all of thick albumen separate)	3	18.75	2	12.50	11	68.75	16	13.56
Total	43	36.44	28	23.73	47	39.83	118	100.00

It should be borne in mind that the structure of the egg can not be used to measure the time between ovulations. It only registers the level of the oviduct where the two eggs come together. Further, the author[1] has called attention to the fact that it is necessary to assume a difference in the rate of the passage of the two eggs through the oviduct, in order to account for the union of two yolks which did not enter simultaneously. Nevertheless, it seems certain that in cases where the eggs do not unite at the upper end of the duct the time of entrance of the two yolks must have been separated by a measurable period.

Table II also shows that some of each type of double-yolked eggs are produced as single eggs, some on the day after a normal egg was laid, and some on a day followed but not preceded by a normal egg. About one-third of the eggs of both type I and type II were produced in each of the three relations to the production of normal eggs, but more than two-thirds of the eggs of type III were single eggs.

This suggests that, while eggs of all types, including type III, may result from a heightened rate of fecundity, the most usual cause of the doubling of eggs at the end of the albumen portion is an abnormal delay of the first egg in the oviduct.

In this section it has been shown, first, that there is a certain, though small, percentage of the cases of double-yolked eggs in which, judging from the egg structure, the two yolks probably entered the oviduct practically simultaneously, and, second, that there is a still larger percentage of cases where, as shown by the egg records of the bird, the two ovulations must have occurred within a few (three or four) hours. It seems certain that in all cases of the simultaneous ovulation of two yolks the complete set of egg envelopes is common to the two. Yet neither the structure of the egg nor the egg record of the bird can prove absolutely that the time between ovulations has not been considerably reduced.

OVARIAN RELATION OF THE TWO FOLLICLES WHICH FURNISH THE YOLKS FOR A DOUBLE-YOLKED EGG

Glaser[2] described the pathological ovary of a bird which habitually laid double-yolked eggs. He concluded that in this case the double-yolked eggs arose from follicles secondarily fused. The secondary fusion of follicles resulted in a common blood supply which, when associated with a common state of permeability in the two ova, resulted in their simultaneous maturity and discharge. That the condition at autopsy of the ovary described by Glaser warrants the assumption that the secondary fusion of the follicles resulted in a common blood supply which was an important factor in the synchronous maturity and ovulation of yolks may, perhaps, be questioned. But at least it offers a suggestion in regard to the origin of double-yolked eggs which is open to investigation.

[1] Curtis, M. R. Op. cit.

[2] Glaser, Otto. Op. cit.

Some observations have been made at this laboratory upon the relation of the two follicles occurring in normal ovaries which have furnished the yolks for double-yolked eggs.

A young pullet, No. 1825 (1913 chick), laid her first egg at 1.30 p. m. on November 20, 1913. This egg was double-yolked. The bird was killed at 4 p. m. the same afternoon, and the ovary was carefully removed and photographed (Pl. LI, fig. 1). It was perfectly normal with a graduated series of seven enlarging yolks and two distinct follicles. There was no egg in the oviduct. The bases of the two follicles were separated by about 15 mm. of ovarian epithelium, which was covered with small yolks.

Another pullet, No. 8053 (hatched in 1913), laid a double-yolked egg at 3.30 p.m. on November 26, 1913. This was her first egg. At 4.35 p. m. the bird was killed. There was no egg in the oviduct. Two normal separate discharged follicles were present on the ovary (Pl. LI, fig. 2). The bases of the two follicles were quite distinct, although not situated at a great distance from each other. They were supplied by separate arteries from the same branch of the ovarian artery. The ovary was apparently normal in every respect. It contained a series of six enlarging yolks.

In both these cases the only yolks the bird had ever ovulated were the yolks contained in the double-yolked egg. In each case these yolks were from separate follicles which had separate blood supplies.

The study of the structure of the egg and of the egg record of the bird has already led to the conclusion that it is not necessary to assume simultaneous ovulation or even an unusually rapid succession of ovulations, except in a small percentage of the cases of double-yolked egg production. It, therefore, is important to consider these points in a study of the ovarian relation of the follicles.

In the two cases just discussed the double-yolked eggs were both of type II—that is, each yolk was inclosed in an envelope of thick albumen and then the two were inclosed in a very thin common envelope of thick albumen. Plate XLVIII, figure 1, is a reproduction of a photograph of one of these eggs, that of bird No. 1825. The doubling of these eggs had evidently occurred far down in the albumen-secreting region of the oviduct. We should not therefore expect that the two yolks had been simultaneously ovulated.

The cases in which there is good reason for suspecting simultaneous ovulations are those in which the two yolks are inclosed in a complete set of common envelopes. This shows at least that they have traversed the entire length of the oviduct together.

An egg of this type was produced on October 18, 1914, by bird No. 139M (Pl. XLVI, fig. 3). The common chalazal membrane has been partly torn away, in order to demonstrate that the two yolks have separate vitelline membranes. The bird laid a normal egg on October

19 and was killed and examined on the 20th. At the time of autopsy there was an egg in the oviduct. The egg record of this bird from October 1 to 20 is as follows:

October....	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Eggs.....	1	...	1	1	1	1	...	1	1	1	(a)	...	...	1	1	1	...	b 1	1	c 1

a Nested, but did not lay. b Double-yolked egg. c Egg in oviduct at autopsy.

Plate LII, figure 1, shows the ovary of this bird. The ovary is perfectly normal, with a series of six enlarging yolks. As would be expected from the egg record, several discharged follicles were found, ranging in size, as in other normal ovaries, from the large one just discharged to those barely visible. The seven largest can be arranged according to size and associated with the eggs laid from October 14 to 20. Follicle A is much the largest and no doubt furnished the yolk found in the oviduct. Follicle B is next in size and evidently furnished the yolk for the egg laid on the 19th. Follicles C and C′ are practically equal in size and probably furnished the two yolks for the double-yolked egg on the 18th. Follicles D, E, and F continue the decreasing series and probably furnished, respectively, the yolks for the eggs on October 16, 15, and 14. All the other discharged follicles are distinctly smaller.

Every follicle on the ovary was carefully examined for evidence of the fusion of follicles or of a common blood supply. (The bird had been injected with starch solution.) All the follicles on the ovary had separate stalks and each had a single cavity. Follicles B, C, C′, D, and E, in common with all the other follicles in that part of the ovary, were supplied by separate small branches from a single large branch of the ovarian artery.

There is, then, in this case no evidence that the double-yolked egg has arisen from a fusion of follicles or from a common blood supply, although the structure of the egg indicates that the two yolks have passed the full length of the oviduct together.

Since there is evidence of simultaneous ovulations in less than one-sixth of the cases of double-yolked egg production and since even in such a case the two follicles may be quite distinct, two simultaneous ovulations resulting from the fusion of follicles are at least a very unusual cause for the production of a double-yolked egg.

NATURE OF THE FOLLICLE WHICH PRODUCED A LARGE YOLK WITH TWO GERM DISKS

A study of all the abnormal eggs produced at the Maine Experiment Station shows that the doubling of an egg in the ovary is rare. An egg belonging to this class was laid on March 1, 1914, by bird No. 311K. Externally this egg resembled a double-yolked egg. Its weight was 82.25 gm. It contained one single very large yolk (weight 30.12 gm.),

with two large and apparently normal germ disks (Pl. XLVI, fig. 1). There was a thin white line visible only part way around the yolk but passing between the two disks and standing out plainly in this region. The size of the yolk, the presence of two germ disks, and this line were the only evidences of doubling. The vitelline membrane was punctured and the yolk removed. The membrane was washed out carefully with salt solution. It contained a *single* cavity, with no suggestion of doubling, except the slight thickening seen as a white line on the surface of the yolk. The weight of albumen and shell, 44.47 and 7.66 gm., respectively, was comparable to the weight of these parts in a double-yolked egg where the two yolks are separate.

This egg clearly belongs to the first class described by Immerman [1] in which the two yolks are in a single vitelline membrane.

The bird which laid this egg was killed at 11 a. m. on the following day. A normal egg weighing 45.81 gm. was in the shell gland. The yolk of this egg was normal, having a single germ disk. It weighed 17.7 gm., which is about 59 per cent of the weight of the yolk with the two germ disks. The egg contained as yet only a small amount of thin albumen and no visible shell. It had evidently just entered the shell gland. The contained yolk had without doubt been ovulated the morning of the day the bird was killed, or about 48 hours after the ovulation of the yolk with the two germ disks. The egg would not have been laid until the second day following the one on which the large egg was laid.

The ovary of this bird is shown in Plate LII, figure 2. Two of the enlarging yolks have been removed, in order to show the follicles. Only three large follicles were present. Two of these, A and B, were about the same size, A being somewhat larger. The stalk of the third, which was considerably smaller, is seen at C. Either A or B must have furnished the yolk with the two germ disks. This yolk was nearly twice the size of the normal yolk in the oviduct. In the time which elapsed between the two ovulations the follicle which had produced this enormous yolk had been resorbed to practically the size of the one just ovulated. Neither of these follicles, nor in fact any other on the ovary, showed any evidence that it was composed of two fused follicles. If the double yolk arose from the fusion of two oöcytes, it seems probable that this took place at a stage earlier than the formation of the follicle.

The practically double size of the yolk contained in a single follicle but with two germ disks suggests that the germinal vesicle may be an important factor in determining the quantity of yolk deposited within a yolk membrane.

In the domestic fowl the fusion of follicles in an ovary capable of producing normal yolks must be of rare occurrence. The examination of several hundred ovaries of laying birds has not furnished a single case.

[1] Immerman, Ferdinand. Über Doppeleier beim Huhn. 43 p., 5 fig. Basel, 1899. Inaugural Dissertation.

In cases of general peritonitis accompanied by visceral adhesions, slight superficial adhesions are sometimes found between the follicles containing hardened yolk. These follicles could never produce normal yolks.

DESCRIPTION OF A FUSION OF YOLKS WHICH MAY HAVE ARISEN FROM A FUSED FOLLICLE

A small egg weighing only 19.88 gm. was laid on October 19, 1913, by bird No. 19K. This egg contained the double yolk shown in Plate XLVI, figure 2, and weighed only 1.45 gm. Neither part had a visible germ disk. The vitelline membranes were fused at the point of contact and there was a communication between the cavities of the two yolks, if they were actually separate yolks. The bird had been laying normal eggs and continued to do so. Why this very immature pair of yolks was ovulated is difficult to understand. Since the bird was not killed, the nature of the follicle which furnished them is not known.

This and the preceding case are the only ones which have come under our observation in which the two yolks were inclosed in a common vitelline membrane.

CONCLUSIONS

The various kinds of evidence given in this paper lead to the conclusion, first, that double-yolked eggs sometimes represent a heightened rate of fecundity and sometimes an abnormally low physiological tone of the oviduct; second, that, even in cases in which the rate of fecundity is high, the ovulations are not always simultaneous; third, from the above it is apparent that the production of a double-yolked egg can seldom be explained as a result of simultaneous ovulations; and, fourth, in cases in which we have the best of reasons for suspecting simultaneous ovulations, the two follicles may be quite distinct.

It seems quite possible that a heightened rate of fecundity may result in every conceivable shortening of the period between ovulations consistent with the daily rhythm in the general physiological activities of the bird. Whether it results in the formation of a double-yolked egg is no doubt determined by the actual length of the period and the following response of the oviduct.

SUMMARY

(1) Double-yolked eggs with normal separate yolks may have all the egg envelopes common to the two yolks, or they may have some separate and some common envelopes.

(2) They may be classified with reasonable accuracy into three groups:

Type I.—Double-yolked eggs having the entire set of egg envelopes common to the two yolks.

Type II.—Double-yolked eggs having separate chalaziferous layers but all or part of the thick albumen common to the two yolks.

Type III.—Double-yolked eggs in which the yolks have entirely separate thick albumen envelopes but a common egg membrane and shell.

(3) Of the eggs studied 16.03 per cent belonged to type I, 70.99 per cent to type II, and 12.98 per cent to type III.

(4) A large series of double-yolked eggs show all gradations within and between these groups.

(5) The most probable interpretation of this phenomenon is that the two components unite at any level of the oviduct from the funnel mouth to the isthmus ring.

(6) The conclusion that the union of the component eggs occurs *indiscriminately* at all levels of the oviduct is strongly supported by the fact that the percentage of eggs of each type is closely porportional to the percentage of the portion of the duct in which the union of two eggs would give double-yolked eggs of that type.

(7) In 36.44 per cent of the double-yolked eggs the ovulations which furnished the two yolks must have been separated by an abnormally short interval, since a normal egg had been laid on the preceding day.

(8) An examination of the egg structure, however, shows that the two yolks have passed the entire length of the duct together in only 16.28 per cent of the cases in which the ovulations are known to have been usually rapid.

(9) While a heightened rate of fecundity may result in the production of an egg of any of the three types, 68.75 per cent of the eggs of type III are single eggs. It seems probable that many of them have resulted from the delay of the first egg in the oviduct.

(10) The ovary of each pullet which had just laid a double-yolked egg as her first egg contained two normal separate follicles which had separate blood supplies. In these cases, however, the doubling of the egg had occurred near the end of the albumen-secreting region.

(11) In a case in which there was evidence from the structure of the egg that the two yolks had passed the entire length of the oviduct together the two follicles were also quite distinct, with separate blood supplies.

(12) This, together with the fact that in only a small percentage of double-yolked eggs is there any evidence of simultaneous ovulation, indicates that the fusion of follicles and a resulting common blood supply is by no means the usual cause for the production of a double-yolked egg.

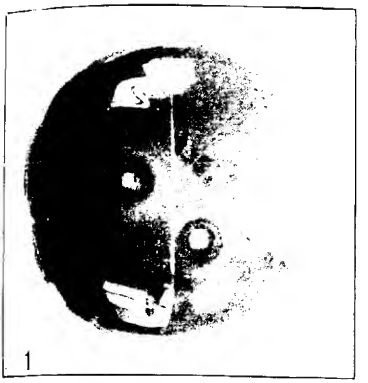
(13) A simple normal follicle furnished the yolk with two germ disks; hence, the fusion of the oöcytes (if this was the origin of the two germ disks) must have occurred before the formation of the follicle.

PLATE XLVI

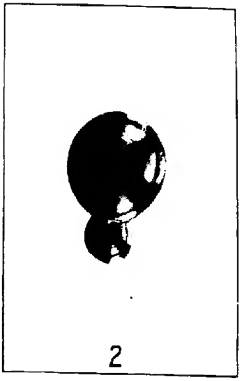
Fig. 1.—Large yolk (weight, 30.12 gm.) with two germ disks; found in a large hen's egg.

Fig. 2.—Fused immature yolks (weight, 1.45 gm.); found in a small hen's egg.

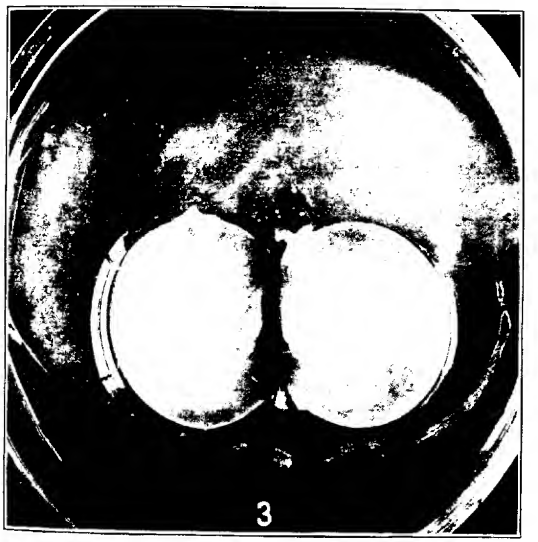
Fig. 3.—Type I double-yolked egg, showing two yolks with separate vitelline membranes but inclosed in a common chalaziferous layer. The yolks were slightly pressed apart before photographing, in order to show that the vitelline membranes were entirely separate.



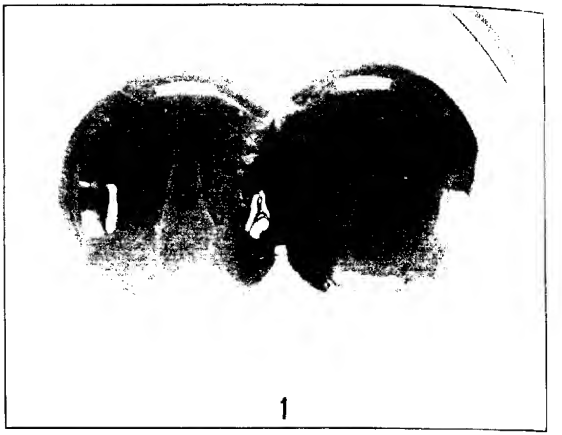
1



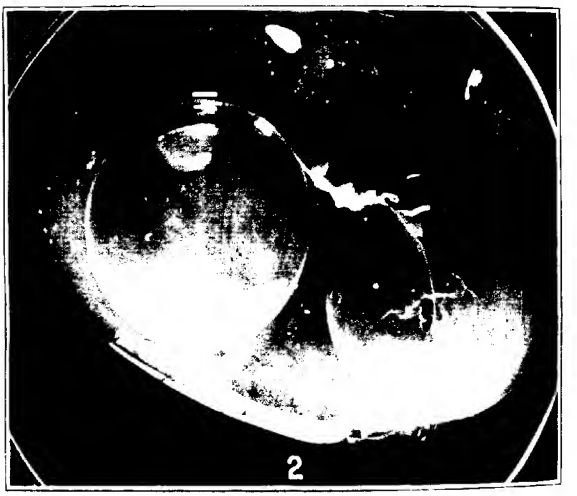
2



3



1



2

PLATE XLVII

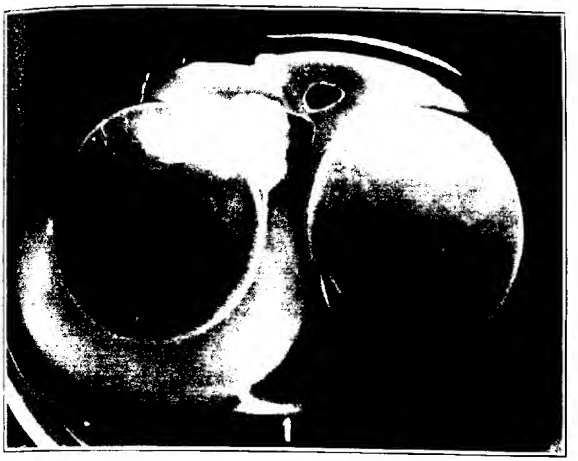
Fig. 1.—Type I double-yolked egg, showing two yolks with separate vitelline membranes but inclosed in a common chalaziferous layer. The yolks were slightly pressed apart before photographing, in order to show that the vitelline membranes were entirely separate.

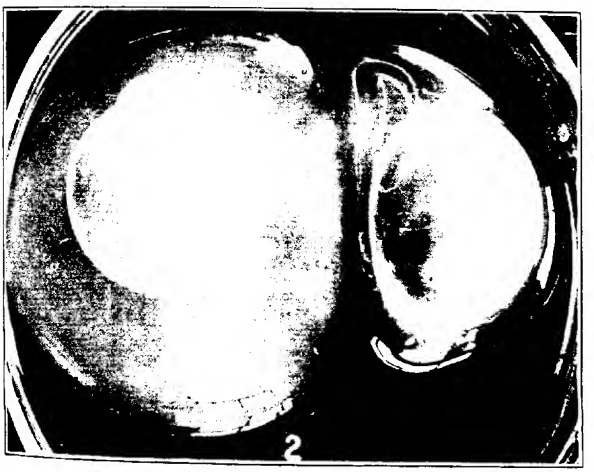
Fig. 2.—Type II double-yolked egg, showing two yolks with separate chalazal membranes but common thick albumen.

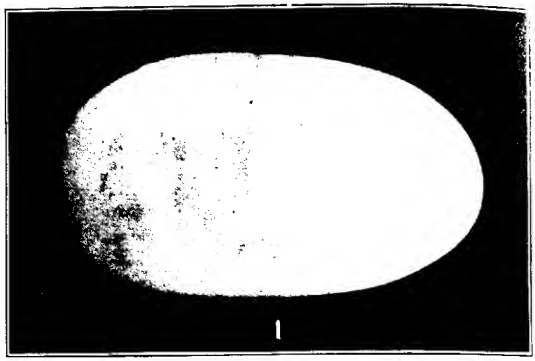
PLATE XLVIII

Fig. 1.—Type II double-yolked egg, showing two yolks with some separate and some common thick albumen envelopes.

Fig. 2.—Type III double-yolked egg, showing two yolks with all the thick albumen separate.


1


2


1


2

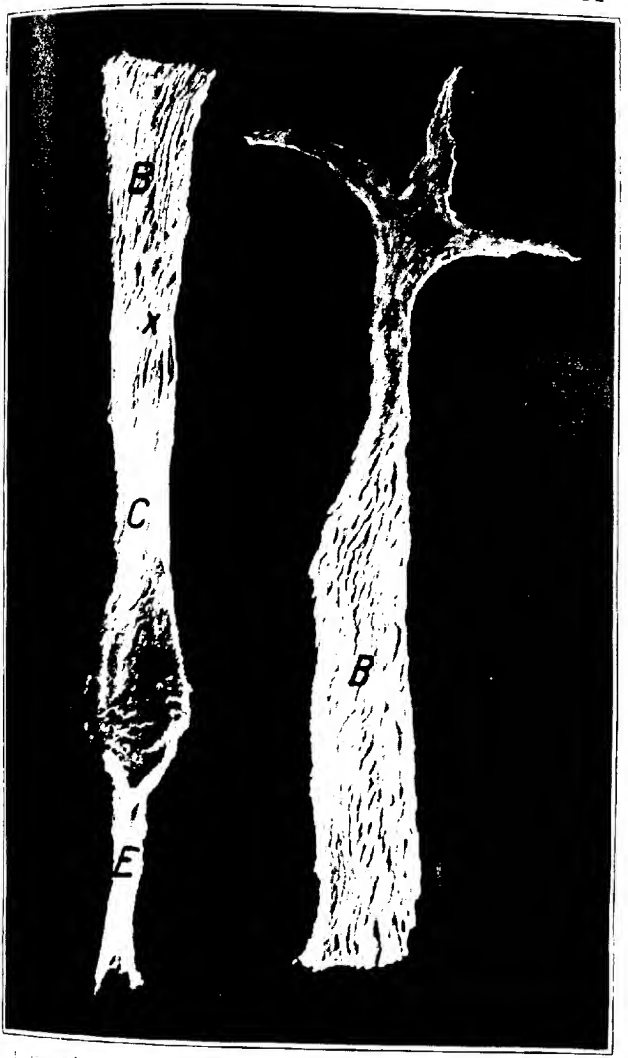
PLATE XLIX

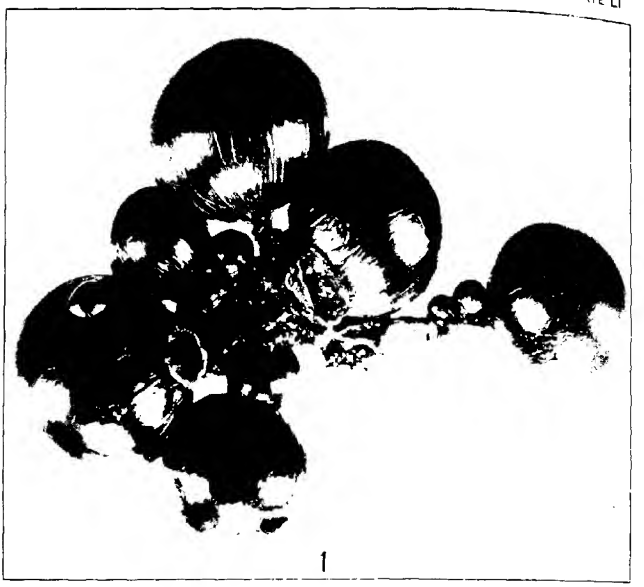
Fig. 1.—Shell of type III double-yolked egg, which shows external evidence of its double nature by a seam in the shell.

Fig. 2.—The inside of the shell shown in figure 1, showing the fold of egg membrane which projected between the two component eggs.

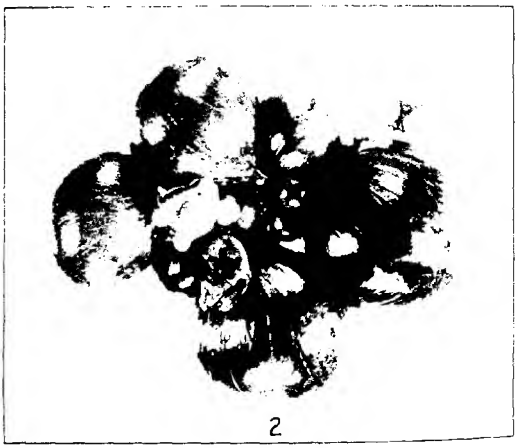
PLATE L

Oviduct removed from a laying bird and cut open along the point of attachment of the ventral ligament. It is opened back, showing the characteristic glandular regions. *A*, Funnel; *B*, albumen-secreting region; *N*, isthmus ring; *C*, isthmus; *D*, shell gland; and *E*, vagina.


B
x
C
E
B



1



2

PLATE LI

Fig. 1.—Ovary of a pullet, showing the follicles which produced the yolks for the double-yolked egg shown in Plate XLVIII, figure 1.

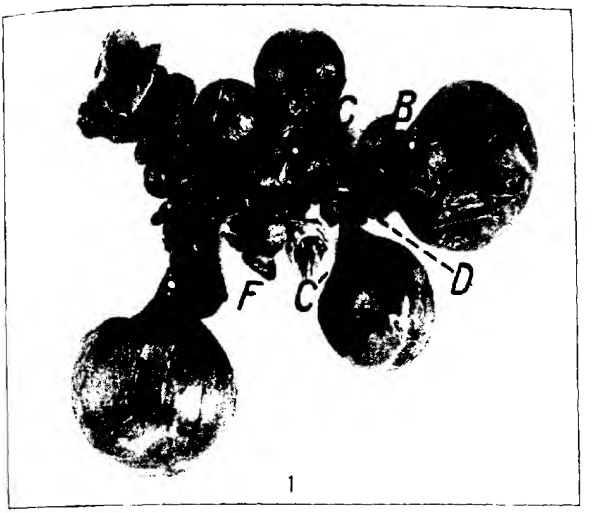
Fig. 2.—Ovary of a pullet, showing follicles which produced the yolks for a double-yolked egg similar in structure to the one shown in Plate XLVIII, figure 2.

75012° -15——3

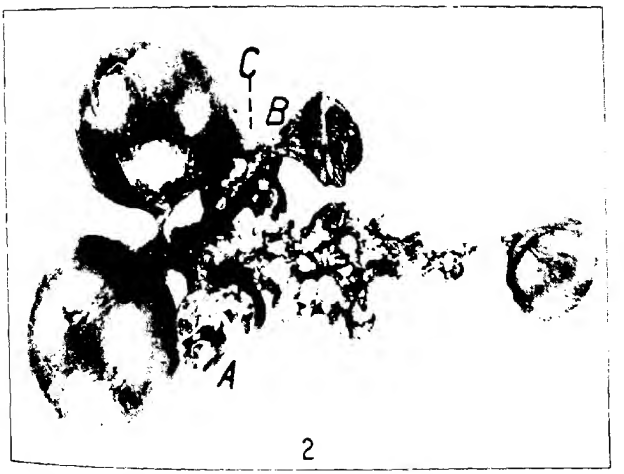
PLATE LII

Fig. 1.—Ovary of a pullet, showing a series of resorbing follicles, two of which (probably C' and C'') produced the yolks for the double-yolked egg shown in Plate XLVI, figure 3.

Fig. 2.—Ovary of a bird, showing the two largest resorbing follicles, one of which produced the yolk with two germ disks shown in Plate XLVI, figure 1.



1



2

BRACHYSM, A HEREDITARY DEFORMITY OF COTTON AND OTHER PLANTS

By O. F. Cook,

Bionomist in Charge of Acclimatization and Adaptation of Crop Plants and Cotton-Breeding Investigations, Bureau of Plant Industry

The word "brachysm" is suggested as a name for abnormal variations of plants characterized by shortening of the internodes, without corresponding reductions of other parts. Brachysm is to be distinguished from nanism, or true dwarfing, which involves proportional diminutions of many parts, if not of all. Genuine dwarfs, with consistent reductions of all of the organs of the plant, would have little value for agricultural purposes, but many brachytic varieties are highly prized. In spite of their shorter internodes, brachytic varieties often bear leaves, flowers, and fruits as large as plants of normal stature, and sometimes larger. Most of the so-called "dwarf," or "bush," varieties of peas, beans, squashes, tomatoes, and other garden vegetables represent brachysm rather than true dwarfing. The "cluster" and "limbless" varieties of cotton belong to the same category, and the San Ramon coffee of Costa Rica affords another example of brachysm in a woody plant.

A special interest may be claimed for the cluster and limbless varieties of cotton because they seem to throw light on the nature of brachysm and similar abnormalities as phenomena of heredity. As brachytic varieties arise by mutation and show alternative inheritance in crosses, they illustrate two of the phenomena of heredity that have received much attention in recent years. The morphological and physiological relations of such characters must be understood before it is possible to appreciate their practical importance in breeding or their bearing upon general evolutionary problems.

SPECIAL FEATURES OF BRACHYSM IN COTTON

The special interest that attaches to the phenomena of brachysm in cotton arises from two general facts: That the shortening of the internodes is usually confined to the fruiting branches and that it is usually accompanied by other abnormalities. On account of the specialized structure of the cotton plant, it becomes possible to learn more of the relations of brachysm to other phenomena of heredity than if all of the internodes of the plant were affected and the other organs remained unchanged.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. III, No. 1
Feb. 15, 1915
G—40

The cotton plant has a definite dimorphism of branches, no flowers or bolls being produced on the main stalk or the vegetative branches.[1] The main stalk and the vegetative branches of cluster cottons are not shortened, but are often longer in brachytic varieties than in those that have normal fruiting branches. The leaves of the main stalk and vegetative branches of cluster cottons are of normal form, but they are larger, of thicker texture, and have longer petioles than those of normal long-jointed varieties. The axillary buds of noncluster varieties usually remain dormant or produce long vegetative branches, but in cluster cottons they commonly develop into short branches and produce one or two bolls. These differences may be considered as direct results of the failure of the fruiting branches to make normal growth, for similar changes occur in noncluster varieties, in plants that have been severely pruned, and also in seedlings that lose their terminal buds through insect injuries or by abortion.[2]

The abnormalities that accompany the shortening of the fruiting branches in cotton are shown in the forms of the leaves and the involucral bracts. The leaves of short-jointed fruiting branches often become smaller and more bractlike, while the bracts are often enlarged and leaflike, and show definite indications of the stipular and foliar elements that are completely fused in normal bracts.

Each of the involucral bracts of a cotton plant represents a modified leaf. The specialized form of the bract results from having the stipules of the leaf greatly enlarged, the petiole entirely suppressed, and the blade reduced and united with the enlarged stipules. The abnormal leaflike bracts and bractlike leaves of cluster cottons show all the stages between normal leaves and normal bracts. In such abnormalities there is usually an obvious relation between the reduction of the blade or the suppression of the lobes of the blade and the enlargement of the stipules of the same leaf, and also between abnormalities of the leaf and of the involucral bracts of the same internode. This can be understood by comparing Plate LIII, which shows an abnormal reduced leaf and an abnormal enlarged bract, with Plate LIV, which shows a leaf and a bract of normal size and proportions. The converse relation is that when the bracts take a more leaf-like form, with an enlargement of the middle or blade element of the bract, it is almost always accompanied by a reduction of the stipular elements, as shown in the leaf-like bract in Plate LIII. When one side of a leaf is reduced or has the lobe sup-

[1] The dimorphic specializations of the branches and leaves of the cotton plant have already been described. (Cook, O. F. Dimorphic branches in tropical crop plants ... U. S. Dept. Agr. Bur. Plant Indus. Bul. 198, 64 p., 9 fig., 7 pl. 1911. Cook, O. F. Dimorphic leaves of cotton and allied plants in relation to heredity. U. S. Dept. Agr. Bur. Plant Indus. Bul. 221, 59 p., 18 fig., 5 pl. 1911.)

[2] Abortion of terminal buds is a frequent result of a peculiar disorder of cotton seedlings previously described. (Cook, O. F. Leaf-cut, or tumosis, a disorder of cotton seedlings. *In* U. S. Dept. Agr. Bur. Plant Indus. Circ. 122, p. 97–111, 1 fig. 1913.)

pressed, as in Plate I,V, the stipule on the same side of the leaf shows a corresponding enlargement.[1]

That brachysm in cotton is confined to the fruiting branches is doubtless connected with the fact that the fruiting branches have more direct relations with the floral organs. On account of the definite dimorphism of the branches of the cotton plant, no floral buds are produced on the main stalks or the vegetative branches. A study of brachysm in cotton seems to indicate that such variations represent intermediate stages or combinations of floral and vegetative characters. Brachytic varieties have leaves that are more like involucral bracts, and bracts that are more like leaves than those of normal long-jointed varieties. From this point of view it is easy to understand that leaves of the fruiting branches would be more likely to show abnormal anticipations of the characters of the floral bracts than the leaves of the vegetative branches or the main stalks, for these have no floral buds and represent earlier stages in the development of the plant.

INDEPENDENT ORIGINS OF BRACHYTIC VARIATIONS

It has been supposed that all of the "cluster" varieties of cotton belong to the same botanical series, but in reality the possession of short-jointed fruiting branches is not a reason for supposing that two varieties are related. Brachytic variations are very frequent and have been found in so many different species and varieties of cotton that the idea of derivation by crossing with a brachytic ancestral type is unwarranted.

Brachysm is not known as a normal character of any wild species of cotton, but seems to follow as one of the results of domestication and selection. The same is apparently true in other families of plants. Brachytic variations have arisen independently in several different types of peas, beans, squashes, melons, and other climbing and creeping plants. It is in such plants that the abnormal nature of brachytic variations is most obvious. Under natural conditions, short-jointed variations of climbing plants would be placed at a still greater disadvantage than those of shrubby plants like cotton or coffee.

If brachytic variations be supposed to represent the formation of a new character, as assumed in the mutation theory of De Vries, it is difficult to understand why the same character should arise suddenly in so many different and unrelated types of plants. But if brachysm be considered as a defect or failure of normal heredity, it becomes easier to understand that many kinds of plants might be subject to similar derangements. That the brachytic tendencies should appear independently in so many different genera and families of plants makes it reasonable to look for a general interpretation of this class of abnormal variations.

[1] A further account of these abnormalities, with figures of some of the intermediate forms of bracts and leaves, may be found in an earlier bulletin. (Cook, O. F. Heredity and cotton breeding. U. S. Dept. Agr. Bur. Plant Indus. Bul. 256, p. 69–78, fig. 3–12, pl. 4–6. 1912.)

DIFFERENT DEGREES OF BRACHYSM

The interest of brachytic variations as a phenomenon of heredity is increased by the fact that the tendency is manifested in many different degrees. The most extreme form of brachysm is represented by a complete abortion of the fruiting branches, but there is an apparently complete series of stages from these completely sterile monstrosities to the normal long-jointed forms. The strictly cluster or limbless forms have the fruiting branches reduced to a single joint or a few very short joints, as shown in Plate LVI. From this extreme condition the intermediate stages run through the ordinary cluster and semicluster types to those that show no reduction of the joints of the fruiting branches. Even in the same field of cotton it is often possible to find a wide range of variations in the lengths of the internodes of the fruiting branches, especially in varieties like the King, that produce many brachytic variations.

In some varieties, such as the Triumph cotton of Texas, the lower fruiting branches often show a tendency to brachysm not shared by branches farther up. Other varieties show the opposite tendency to form long-jointed fruiting branches on the lower part of the stalk and short-jointed branches on the upper part. Some varieties that usually have long-jointed fruiting branches show the cluster tendency when the growth of the plants is restricted by unfavorable conditions, while other varieties are always short-jointed. There may also be pronounced irregularities in the lengths of the joints, even on the same fruiting branch. (See Pls. LVII and LVIII.)

In addition to the fundamental difference between the vegetative and fruiting branches, the basal internode of the fruiting branches is generally longer than the others and often maintains its length when the others are shortened. In a peculiar variety of Egyptian cotton grown in experimental plantings at Bard, Cal., under the name of Dale, most of the fruiting branches develop only this long basal internode, and produce only a single boll, the other internodes being aborted. (See Pl. LXII.) When the leaves, buds, and joints of such branches are suppressed, the boll appears to be borne on a greatly elongated pedicel.

The extent to which these abnormalities are often carried may be more easily understood by reference to the photographs of the plant shown in Plate LXI, figure 1. The main stalk was rendered completely sterile by the abortion of all of the fruiting branches, though the vegetative branches of the same plant produced a few fruiting branches and ripened a few bolls. Finally the growth of the main stalk was stopped by the abortion of the terminal bud. (See Pl. LXI, fig. 2.) In more normal plants of this variety the single-jointed fruiting branches are often accompanied by one or two other branches of similar form arising from the axillary bud. (See Pl. LXII.) In an extreme case like that shown in Plate LXI, the axillary buds abort, as well as the extra-axillary buds

that normally produce the fruiting branches. The regular abortion of these axillary buds renders it the more probable that the abortion of the terminal bud was not accidental. The case shown on the left-hand figure of Plate LXII, the transformation of the terminal bud into a flower bud subtended by an abnormal leaf, affords still more definite evidence of abnormality.

SHORTENING OF INTERNODES BY DROUGHT

The internodes are always longer under conditions that favor luxuriant growth of the plants than where growth is restricted by drought. Though this relation is general, some varieties shorten their internodes much more than others. The susceptibility to shortening is sometimes so great that the same variety may be short-jointed like a cluster cotton in some places, while under other conditions it behaves as a normal long-jointed variety. Attention was called some years ago to a case of this kind in a variety called the Parker that had been grown in Texas for several seasons as a long-jointed variety, but behaved as a short-jointed or semi-cluster variety at Del Rio in the season of 1907.[1]

The difference between the true brachysm and this false brachysm induced by changes of external conditions is that the latter is not inherited. The false brachysm represents an adaptive change or accommodation to the conditions instead of a definite alteration of the expression relations of the characters. When whole stocks of plants or animals respond in the same way to a change of external conditions, the changes are usually in the nature of accommodations and are readily reversible. But the possibility that an increase in the number of heritable variations toward brachysm might be induced by environmental shortening of the fruiting branches would be worthy of investigation. This possibility is suggested by the fact that individual variations in the direction of the cluster habit appear more frequently in some localities than in others in the same variety of cotton. Thus, it was noticed in the season of 1913 that plants of the cluster form were of frequent occurrence in many fields of Durango cotton in the Imperial Valley of California, whereas in a field of Durango cotton at Deep Creek, Va., no cluster plants could be found.

Other illustrations of the influence of external conditions are afforded by irregularities in the lengths of the internodes of the same plant, or even of the same branch. (See Pl. LVII and LVIII.) In such cases the

[1] "The Parker variety of cotton showed a pronounced semi-cluster habit or shortening of the internodes of the fruit branches, a notable departure from the previous behavior of our stock of this variety, which had been under observation in several different localities in the preceding years. Apart from the fact that every precaution is taken to avoid mistakes in labeling and planting the seed, the possibility of error in this case seems to be entirely eliminated by the fact that six different selections of Parker were grown at Del Rio and that all of them behaved in the same manner with reference to this change in the lengths of the fruiting branches. Plantings of the same strains from the same lots of seeds in several other localities in Texas in the same season produced no such results." Cook, O. F. Suppressed and intensified characters in cotton hybrids. U. S. Dept. Agr. Bur. Plant Indus. Bul. [illegible] 1909.

environmental shortening or false brachysm is often accompanied by other abnormalities, like the inherited form of brachysm. On a short internode intercalated between two of normal length the pedicel is likely to have a sloping or decurrent base, with the end of the internode prolonged beyond the insertion of the pedicel, though usually not so much as in cluster cottons.

This irregularity is less difficult to understand when it is remembered that the floral bud of each internode develops in advance of the growth of the next internode. On account of this sequence of development, two structural elements may be recognized in the internodes, the tissues that are developed early to support the pedicel and those that develop somewhat later in connection with the next internode. If a change to more favorable external conditions occurred during the growth of an internode, its effect must be greater upon the part of the internode that is the last to reach its full development. Thus, in connection with the development of a longer internode next to a short one, a slight elongation of the supporting part of the short internode would be induced, and a resulting tension between the two sides of the internode. This would account for the tearing of the tissues at the base of the pedicel, which often occurs. Hence, we see that a part of the abnormality of the internodes that accompanies brachysm may be capable of a simple mechanical explanation, as arising from changes in external conditions during the period of development of the affected internodes.

RETENTION OF BLASTED BUDS IN BRACHYTIC VARIETIES

The facts of brachysm in cotton seem to indicate that the shortening of the internodes of the fruiting branches is in the nature of a premature or accelerated expression of the floral character. This view seems preferable to the idea that a new character has been substituted for an old one in the mechanism of transmission, as usually assumed in theories of mutation and Mendelism. That the shortened internodes partake of the nature of the pedicels of the flowers is indicated not only by their reduced length but by the fact that they are often completely fused with the pedicels. At the base of a normal pedicel is a joint or layer of specialized tissue indicated externally by the absence of hairs and oil glands from the surface, but in cluster cottons the formation of this specialized layer is irregular. It is one of the recognized peculiarities of cluster cottons that abortive buds and those that are infested by boll-weevil larvæ often remain attached to the plant, whereas in varieties with normal fruiting branches the blasted buds are soon shed. The lack of definite differentiation between the pedicels and the joints of the branches is responsible for the more frequent retention of the buds in cluster varieties.

Normal shedding of the buds takes place by the formation of a circular fissure at the base of the pedicel, the subsequent wilting of the bud, and

the breaking away of the central pith of the joint. The circular fissure is formed just above the slight ridge or rim that connects the base of the stipules, the position being marked in advance, as already stated, by a narrow zone of smooth skin without any of the hairs and oil glands that are scattered over all of the neighboring surfaces.

The less definite differentiation of internodes and pedicels in cluster varieties often interferes with the formation of a normal circular fissure for the shedding of the abortive buds, which then remain hanging on the plant. The lower side of the base of the pedicel, the side that faces the main stalk of the plant, is found to be more or less confluent with the surface of the supporting internode. The zone of smooth tissue that indicates the position of the fissure, instead of being circular, may extend far down on the internode; or all indication of a fissure zone may be lacking, so that the pedicel appears as a direct continuation of the internode. The result of such malformations is that the blasted buds, instead of promptly falling off, turn brown and shrivel while still attached to the plant.

The casual observer is likely to suppose that the shriveled buds have been stricken by a blight, and there is often a strip of dead tissue running down on the internode from the base of the withered bud as though a bacterial or fungous disease were extending along the branch. Though often mistaken for a diseased condition, such injuries are merely the mechanical consequences of the abnormal structure of the internode and the failure to form a properly specialized joint between the internode and the pedicel of the floral bud. The formation of the joint is usually indicated, for the death of the epidermis commonly follows a definite line, even when the bud does not drop off. A complete separation of the underlying tissues allows the shriveled bud to fall away eventually, leaving a long scar extending down the internode, instead of a normal circular scar at the end of the internode. (See Pl. LVIII.)

Decurrent pedicels are not confined to brachytic varieties, but are often found in abnormal individuals of long-jointed varieties, though usually the internodes that have the decurrent pedicels are shorter, and other indications of abnormality may be present. Thus, in connection with the examples of decurrent pedicels shown in Plate LVIII there is an abnormal inequality in the lengths of the internodes, one being about five times as long as the others.[1]

MORPHOLOGY OF DECURRENT PEDICELS

As already noted, extreme cases are sometimes found in which the pedicels are not only decurrent upon the internodes but seem to lose their

[1] A somewhat different interpretation of the decurrent pedicels of the cotton plant is presented by Prof. Francis E. Lloyd. (Lloyd, F. E. Abscission. *In* Ottawa Nat., v. 28, no. 3/4, p. 41–52, 3 fig.; no. 5/6, p. 61–75. 1914.)

terminal positions and to arise from intermediate points. Examination of such cases at first suggested the idea that the floral bud might belong morphologically to the next internode below. In this view the pedicel would be merely coalesced with the supporting internode, instead of being produced from it. The branch morphology of the cotton plant is unusually complicated on account of the dimorphism of the branches and the extra-axillary position of the floral buds and of the buds that give rise to the fruiting branches.[1]

The assignment of the floral bud to the internode below seems to be forbidden by the fact that when the base of the pedicel becomes decurrent upon the supporting internode the stipule that subtends the pedicel and the stipular rim that incloses the base of the pedicel also become elongated and decurrent along the side of the internode. Thus, instead of merely a lower insertion of the flower bud, the whole internode is modified, and the nature of the modification makes it clear that the floral bud is borne normally above the stipular rim.

Though other families of plants afford instances where flower stalks or floral branches remain united with the basal portion of the next internode, it is very difficult to believe that an adnate or coalesced pedicel would be able to surmount the stipular rim and climb, so to speak, into the axil of the leaf of the internode with which it had become coalesced. In rare cases the pedicel of a cotton boll is joined to the internode above, but the result is clearly abnormal, and lends no support to the theory of coalescence of pedicels and internodes as a normal condition. (See Pl. LX.) A pedicel that is united with an internode is usually longer than the normal pedicels, while the internode is shorter than the others and is turned from its normal position to follow the direction of the pedicel to which it is attached. There is no tendency in these adherent pedicels to form an angle or a joint at the end of the internode, as might be expected if the theory of coalescence were correct.

The presence of a flower bud on the basal internode of fruiting branches offers another difficulty under the theory of coalescence. If it were assumed that each floral bud belongs, not to the supporting internode, but to the one lower down, it would necessarily follow that the floral bud of the basal joint of a fruiting branch could not belong morphologically to the fruiting branch at all, but must be assigned to the main stalk or the vegetative branch from which the fruiting branch is produced. This supposition would add new elements of complexity to the structural morphology of the cotton plant, already sufficiently complicated.

BRACHYSM ACCOMPANIED BY FASCIATION AND ADHESION

Further reasons for looking upon brachysm as a failure of normal differentiation of parts are found in the other abnormalities of the

[1] Cook, O. F. Morphology of cotton branches. *In* U. S. Dept. Agr. Bur. Plant Indus. Circ. 100, p. 11-16. 1911.

branches that often accompany brachytic variations and are of very frequent occurrence in cluster varieties. Fasciation, or duplication of parts, appears in many different stages, ranging from simple forking of internodes or pedicels to inclusion of two flowers in the same involucre, or two bolls in the same calyx, or to an abnormal increase of the number of carpels.[1]

Sometimes the fasciated internodes remain united for their whole length and bear two leaves at the end, like the internodes of opposite-leaved plants. In rare cases double internodes bear two flowers or bolls, though usually the floral buds of abdominal internodes are aborted. (See Pl. LIX.)

Union between the pedicel of a boll and the next internode of the fruiting branch is another abnormality to which reference has already been made. Adhesion occurs in connection with brachysm, though it also appears occasionally in connection with short joints in noncluster varieties. It is more likely to be noticed in such cases because of the contrast with the normal joints of the branch. Doubtless as a result of the fact that the pedicel of the floral bud develops normally in advance of the next joint of the branch, these adherent internodes are bent upward in the direction of the pedicels to which they are joined and form a distinct angle or elbow with the preceding internode of the branch. (See Pl. LX.) It is evident in such cases that the abnormality involves something more than a mere adhesion of the epidermal tissues, for the affected internodes are much shorter than the others, and even shorter than the pedicel to which they are united. In most cases the short joints lose their floral buds or young bolls by abortion.

ANALOGY BETWEEN BRACHYTIC VARIATIONS AND HYBRIDS

The fact that the shortening of the internodes is so often accompanied by abnormal leaves and involucres suggested the view here advanced, that brachysm represents a failure to maintain the normal specializations of the parts. Considered as examples of intermediate expression of characters, the shortened internodes and abnormal involucres of the cluster cottons afford a suggestive analogy with the abnormal intermediate forms of branches and involucres that often appear in hybrids between diverse species of cotton. This analogy may be supported by the fact that sterility, or blasting of the buds or the young bolls, is very frequent in brachytic varieties of cotton, as well as in hybrids, and is especially common in involucres that have the abnormal intermediate forms of bracts.

The idea that short-jointed variations differ from the parent stocks in only this one character, as often assumed by writers on Mendelism, is

[1] Reasons for looking upon fasciation as a symptom of degeneration have been given by Thomas Meehan. (Science, v. 3, no. 70, p. 694. 1884.) Meehan concluded that "a fasciated branch is an imperfect and precocious attempt to enter on the flowering or reproductive stage."

evidently not true of brachytic variations of cotton. While it may be that the mutations of other plants do not show changes in so many characters in connection with brachysm, the general fact seems to be that mutative changes affect several characters at once, instead of one character alone. It is possible, of course, to disregard the other differences and thus simplify the discussion of the Mendelian phenomena by giving exclusive attention to single differences, but many of the statements that have been made give a very misleading idea of the nature of mutative variations.[1]

In normal cotton plants there are sharp contrasts in length between the internodes that form the joints of the fruiting branches and those that form the pedicels of the flowers, just as there are definite differences in form and structure between the foliage leaves and the specialized leaves that constitute the involucre. In brachytic varieties these contrasts become less marked. The changes of characters are all in the direction of reduced specialization of internodes. Instead of the normally contrasted expression of the characters of the different kinds of internodes represented in the normal branches and floral parts, there is a reduced contrast or intermediate expression of the characters, resulting in the formation of abnormal internodes. Variations of this kind, resulting from intermediate expressions of characters that are normally distinct or separate in expression, may be described as metaphanic variations. These would form a general class of abnormalities to include brachysm and other similar aberrations of heredity.

BRACHYSM AND HOMOEOSIS

The nearest approach to a recognition of metaphanic variations as representing intermediate expression of characters, as a general factor in heredity, is probably to be found in the theory of translocation of characters, or homoeosis, brought forward a few years ago by Dr. R. G. Leavitt. The theory of homoeosis is that "a character or a system of organization which has been evolved in one part of the body is transferred, ready-made, tc another part." [2]

The theory of homoeosis might be applied to the phenomena of brachysm in cotton by considering that a partial translocation or homoeosis had taken place, for the characters of the internodes, leaves, and involucral bracts are intermediate. The leaves become more bractlike, the bracts more leaf-like. But to represent typical cases of homoeosis the leaves would need to be replaced by bracts or bracts by leaves, the

[1] "The difference between a tall and a dwarf pea is not the same as the difference between a tall and a dwarf man. In a human dwarf everything is on a smaller scale than in the normal man. But a dwarf pea is not simply a miniature edition, as it were, of a tall one—it differs from a tall pea in one single characteristic, the length of the internodes, i. e., the sections of the stem between two nodes, or joints, where the leaves are given off." Darbishire, A. D. Breeding and the Mendelian Discovery. p. 12-13. London, New York, 1911.

[2] Leavitt, R. G. A vegetative mutant, and the principle of homeosis in plants. *In* Bot. Gaz., v. 47, no. 1, p. 64. 1909.

primary idea being that of transference of characters from one place to another, which is not the same as abnormal or intermediate expression of characters. Leavitt's idea of homoeosis may appear to be in better accord with the Mendelian theory of heredity, which assumes that variations arise from differences in the transmission of characters, but metaphanic variations seem to represent differences in the expression of the characters rather than differences in transmission.

The present interpretation of metaphanic variations is based on the recognition of a definite distinction between transmission and expression as two essentially different processes, though usually described together under the general term "heredity." Characters are often transmitted without being expressed in visible form, as Darwin pointed out. Galton made a formal distinction between patent and latent characters—that is, between characters that are brought into expression and those that are transmitted without being expressed. Latency occurs frequently in connection with Mendelian characters and has received much attention in recent years, but the importance of the distinction between transmission and expression is often overlooked and theories of transmission are often applied to phenomena that belong to the field of expression. With this distinction in mind, the idea suggested by metaphanic variations like brachysm is not that the characters of one part have been transmitted in some irregular manner to another part of the plant, but that the normal sequence of changes or contrasts in the expression of the characters is no longer observed. It is reasonable to believe that all the characters are transmitted to all the parts, including those that usually do not serve the purposes of propagation. Each of the internodes of the cotton plant is capable of reproducing all the other parts. From this point of view the idea of translocation or transfer of characters no longer seems adequate to account for abnormal intermediate characters like brachysm. It seems more reasonable to think of metaphanic variations as arising because the characters are being confused or combined in expression.

In connection with the theory of homoeosis Leavitt suggested that the translocation of characters from one part of the body of a plant or animal to another part opened the way to evolutionary changes, and many examples were given in support of this interpretation. Intermediate expression of the characters, involving a loss of specialization or differentiation of parts, may also be considered as one of the ways in which plants may vary and thus initiate evolutionary changes, but reasons have been given already for looking upon metaphanic variations as negative or degenerative changes of characters rather than as having progressive evolutionary value. Instead of being taken as new characters that are being added to the mechanism of transmission, metaphanic variations may mean that the mechanism of expression has become deranged so that the old characters are not normally developed.

The prevalence of abortion in cluster cottons is one of the most striking evidences of the degenerative nature of such variations. In strongly clustered varieties completely sterile individuals are often found, the result of abortion of all the buds or young bolls or of all the buds which produce fruiting branches. Yet these completely sterile plants are less harmful to the stock than the other less abnormal degenerates that are able to reproduce themselves from seed and also to contaminate their neighbors with inferior pollen. That cluster cottons should seem more unstable than other varieties and more inclined to the production of abnormalities is easier to understand when they are considered as metaphanic variations, representing intermediate expressions of characters that are normally distinct.

AGRICULTURAL DEFECTS OF "CLUSTER" COTTONS

Having considered the general nature of the cluster character of cotton and the accompanying variations, we are in better position to understand the physiological status and practical value of such forms. The first impression of cluster varieties is that they are more fruitful, for they are able to set their buds and bolls more rapidly than varieties with normal fruiting branches. But when we have learned that brachysm is in the nature of a malformation and is frequently accompanied by malformations of leaves and bracts and abortion of both the floral and the vegetative buds, it becomes apparent that the brachytic variants are to be avoided by the breeder, especially when they bear other marks of degeneration.

The limited size and more upright habits of growth make it possible for more of the short-branched plants to stand in the same area, and very large yields may be obtained with favorable conditions of soil and season. But if the conditions prove unfavorable, cluster varieties are likely to suffer worse than others and to produce smaller crops, so that it is very doubtful whether the cluster habit is a practical advantage. The extreme forms of clustering are certainly undesirable, for in such varieties many of the plants are likely to become sterile through the blasting of all of the buds, the tendency to abortion being greatest when unfavorable conditions are encountered during the crop season.

Another agricultural consideration is that the crowding of the bolls together makes picking more difficult. This is especially true, of course, when clustering is accompanied by fasciation and other abnormalities, so that the bolls are malformed or misshapen. The opening of the bolls is also likely to be irregular, because cluster cottons often produce many late bolls on short branches developed from the axils of the leaves of the fruiting branches. Such bolls are usually undersized, as well as late in opening.

A further objection to the cluster character, especially in long-staple cottons, is that the lint of cluster varieties or of individual variations in

the direction of clustering is generally inferior to that of adjacent noncluster plants. This may be due to the fact already noted, the greater susceptibility of cluster plants to unfavorable conditions, owing to their restricted vegetative development. Occasional exceptions have been noticed, where cluster plants produced lint as good or better than that of noncluster neighbors, but there can be no doubt that the general tendency is in the direction of inferiority of lint.

CONCLUSIONS

Brachysm is a term proposed to designate the shortening of the vegetative internodes of plants. It is a hereditary abnormality, indicating degeneracy, that has appeared in independent mutative variations in many distinct families of plants, including many cultivated forms. Brachytic variations are of frequent occurrence in cotton, giving rise to the so-called "cluster" and "limbless" varieties, and afford unusually favorable opportunities for learning the nature and physiological significance of such variations.

The shortening of the internodes of the cotton plant is usually confined to the fruiting branches without affecting the main stalk or the vegetative branches. Brachytic variations occur independently in different species and varieties of cotton and do not constitute a natural group with a common origin.

Brachytic varieties of cotton usually show other abnormalities of the internodes, leaves, and involucral bracts. There is also an increased tendency to abortion of the floral buds, and the blasted buds often remain attached to the plant, because of the absence of well-differentiated abscise-layer at the base of the pedicel.

Though brachytic variations arise by mutative changes in the expression of the characters and show alternative Mendelian forms of inheritance, they afford no additional support to the general theories of mutation and Mendelism as explaining evolution. Such variations represent reduced specialization or intermediate expression of characters and are degenerative in nature. They are not to be considered as examples of normal heredity or of the evolution of new characters. The abnormalities of brachytic variations are analogous to those found among hybrids and are likewise accompanied by tendencies to sterility or abortion of buds.

Brachysm is to be associated with other forms of intermediate expression of characters, representing a general class of metaphanic variations. A more definite recognition of this class of variations is desirable in connection with the investigation of general problems of heredity and evolution.

The agricultural value of brachytic varieties of cotton is impaired by the tendency to abnormal variations and sterility and also by the fact that the cluster cottons are more severely affected by unfavorable conditions. Hence, brachysm is to be avoided in the breeding of superior varieties of cotton.

PLATE LIII

Abnormal simple leaf on fruiting branch of Egyptian cotton, accompanied by abnormal leaf-like bract, remainder of involucre and floral bud removed. Natural size. Photographed by Mr. G. B. Gilbert.

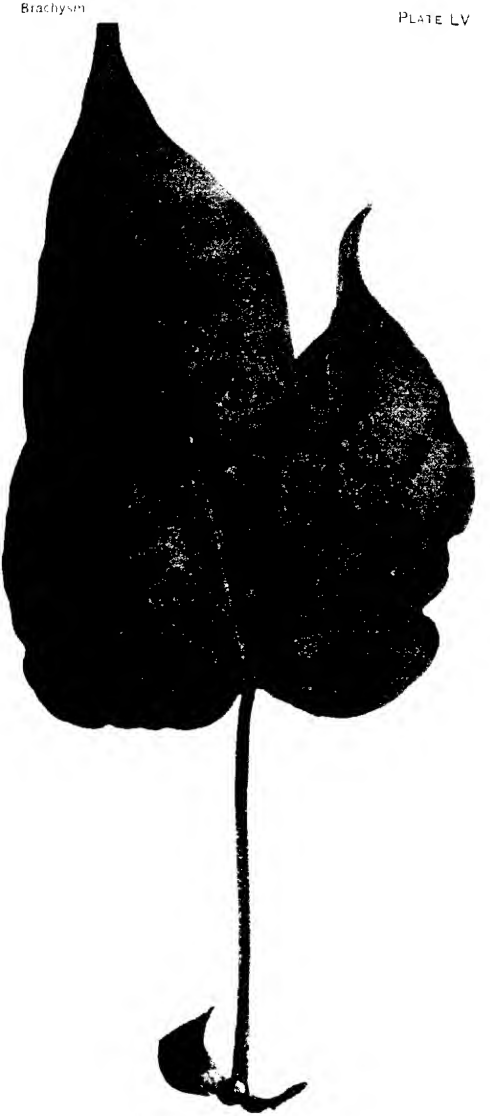
PLATE LIV

Normal 3-lobed leaf of fruiting branch of Egyptian cotton, accompanied by normal involucral bract for comparison with Plate LIII. Natural size. Photographed by Mr. G. B. Gilbert.

75012°—15——4

PLATE LV

Abnormal leaf of fruiting branch of Egyptian cotton with one stipule enlarged and the lobe of the same side wanting. Natural size. Photographed by Mr. G. B. Gilbert. Specimen from Bard, Cal., on October 2, 1913.




1
2

PLATE LVI

Brachytic fruiting branches of "cluster" cotton (Willcts Red Leaf) shortened to a single internode by abortion of terminal bud. Natural size. Photographed by Mr. C. B. Doyle.

Fig. 1.—The boll at the right is borne by a very short branch from an axillary bud.

Fig. 2.—The boll at the right is borne by the shortened fruiting branch. The left-hand boll represents a shortened branch in the axil of the leaf that subtends the fruiting branch.

PLATE LVII

Normal and brachytic joints on same fruiting branch of Upland cotton. Natural size. Photographed by Mr. C. B. Doyle. Specimen from Bard, Cal., on October 13, 1912.

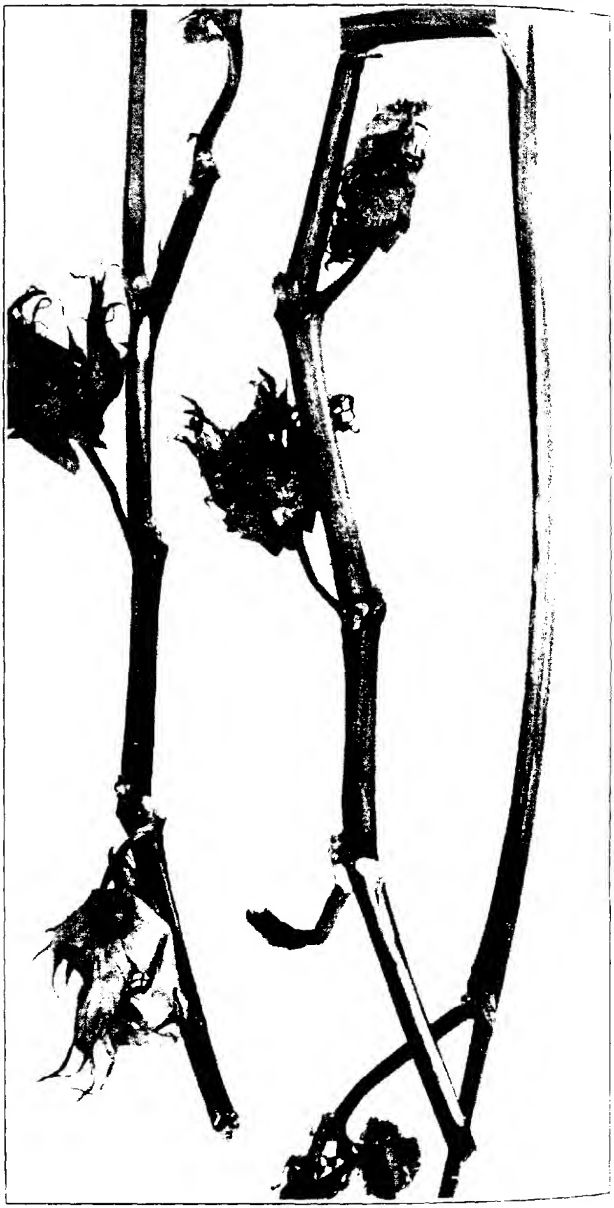
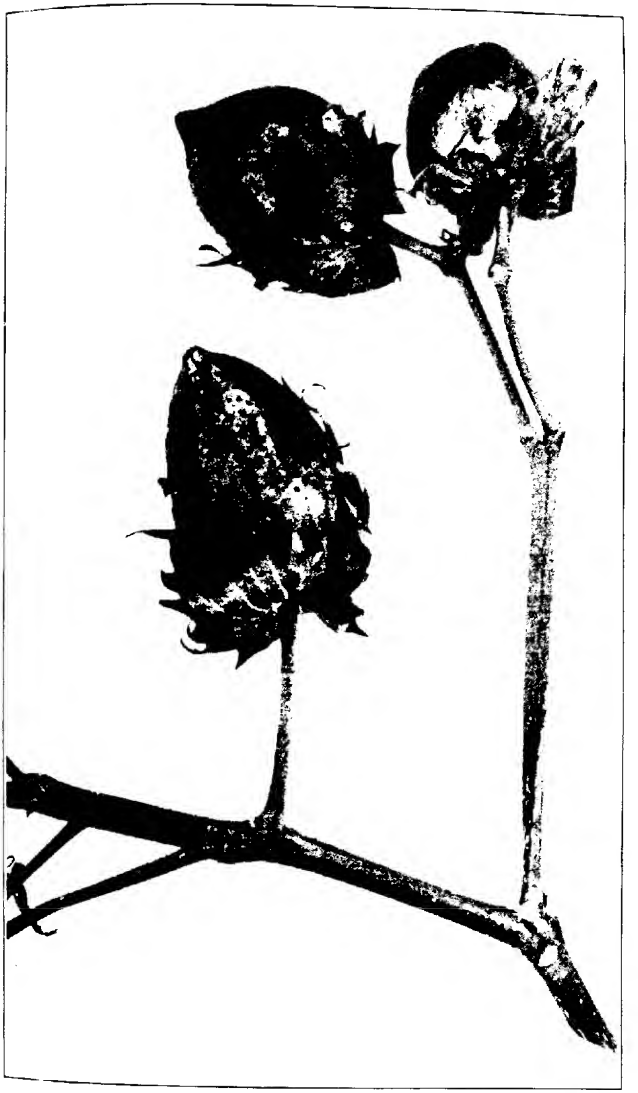


PLATE LVIII

Branches of abnormal variation of Upland cotton, with abortive buds remaining attached to branches by decurrent pedicels and elongated bud scars. The left-hand branch shows abnormal inequality in the lengths of the internodes.

PLATE LIX

Portion of brachytic fruiting branch of Simpkins cotton producing twin fasciated branches from an axillary bud. The boll of the next internode has an elongated, somewhat decurrent base, while that of the third internode of the branch is aborted and shriveled, only the pedicel being shown, at the left of the plate. Natural size. Photographed by Mr. C. B. Doyle.



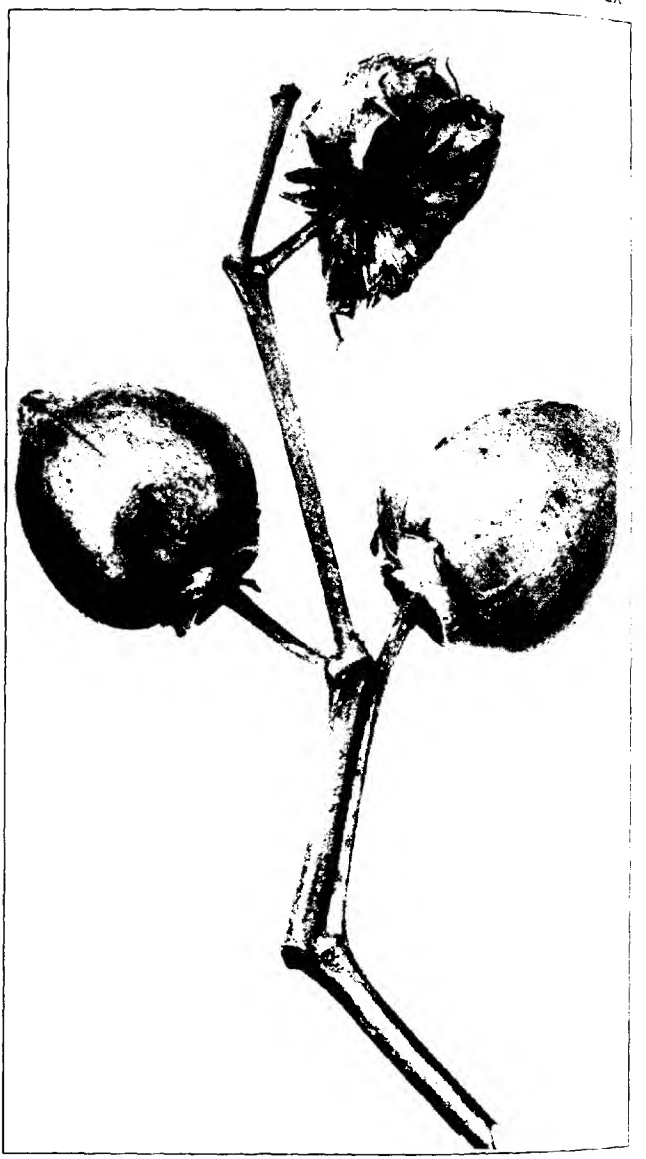


PLATE LX

Portion of fruiting branch of Columbia cotton, with one internode adnate to the pedicel of the boll of the preceding internode. Specimen from Easley, S. C. Natural size. Photographed by Mr. C. B. Doyle.

PLATE LXI

Fig. 1.—Plant of Dale Egyptian cotton, showing complete abortion of fruiting branches on the main stalk, while the vegetative branches of the same plant produced a few fruiting branches and ripened a few bolls. Plant grown at Bard, Cal. Photographed by Mr. C. B. Doyle.

Fig. 2.—End of main stalk of plant shown in figure 1, showing abortion of terminal bud and compensatory thickening of the petioles. Natural size. Photographed by Mr. C. B. Doyle.

PLATE LXI

PLATE LXII

Ends of main stalks of two plants of Dale Egyptian cotton, showing simple fruiting branches and closely similar axillary fruiting branches. In one case the terminal bud was aborted, while in the other it was transformed into a boll subtended by an abnormal bract-like leaf with the stipules enlarged and the blade entire, instead of 5-lobed. Plants grown at Bard, Cal. Photographed by Mr. C. B. Doyle.

ABILITY OF COLON BACILLI TO SURVIVE PASTEURIZATION

By S. HENRY AYERS, *Bacteriologist*, and W. T. JOHNSON, Jr., *Scientific Assistant*, *Dairy Division, Bureau of Animal Industry*

INTRODUCTION

The presence of colon bacilli in pasteurized milk is generally interpreted as meaning either that the milk was not properly heated or that it was reinfected after pasteurization by careless handling. This interpretation is based on the low thermal death point of cultures of *Bacillus coli*.

Van Geuns (4)[1] in 1899 found that the *Bacillus neapolitanus* of Emmerich, the same organism as the *B. coli communis* of Escherich, was destroyed by heating for five minutes at 59° C. (138.2° F.) and one minute at 62.5° C. (144.5° F.). Based on the work of Van Geuns, Ringeling (6) examined 75 samples of pasteurized milk from 24 dairies in Amsterdam for the presence of colon bacilli. In 16 per cent of the samples examined he found *B. coli* present. Since colon bacilli were found in pasteurized milk from 10 of the 24 dairies, Ringeling concluded that this proportion (41 per cent) of the dairies in Amsterdam did not pasteurize or handle the milk properly.

During recent years numerous investigators have studied cultures of *B. coli* and found that the organisms were easily destroyed at temperatures below 60° C. (140° F.), which is the lowest pasteurizing temperature.

In a previous study by the writers (1) of the bacteria which survive pasteurization 19 samples of raw milk in sterile flasks were heated for 30 minutes at 62.8° C. (145° F.). Each sample after pasteurization was examined carefully for the presence of colon bacilli, but none were found.

All these results naturally strengthened the opinion that the presence of *B. coli* in pasteurized milk might be a valuable index as to the efficiency of the process and the care observed in handling the milk after the heating process.

At times, however, it has been found that high temperatures were required to destroy cultures of *B. coli*. Gage and Stoughton (3) in a study of the resistance of *B. coli* to heat found that sometimes cultures were destroyed only by heating to temperatures much higher than the usual thermal death point. Heat-resistant strains have been also found by De Jong and De Graaff (5). Certain strains with which they worked re-

[1] Reference to "Literature cited" is made by number, p. 409.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. III, No. 5
Feb. 15, 1915
A—13

quired 30 minutes heating at 70°–72° C. (158° to 161.6° F.), in order to destroy them. Zelenski (9) also found strains of *B. coli* which resisted high temperatures.

In view of this uncertainty regarding the thermal death point of *B. coli* and its relation to pasteurizing temperatures, the following experimental work concerning the ability of colon bacilli to survive pasteurization was undertaken. The number of cultures used in the experiments was 174.

METHOD OF DETERMINING THE THERMAL DEATH POINT

The colon cultures were grown first in plain, neutral extract broth for 18 hours and then inoculated by means of a small-bore pipette into litmus-milk tubes. Four drops constituted an inoculation in each milk tube. In making the inoculations care was taken not to have any of the culture touch or any of the inoculated milk wash upon the sides of the tube, either during the handling or during the subsequent heating.

The inoculated milk tubes, with the exception of the control tubes, were heated in a water bath in which the water was agitated, and the temperature of the milk was recorded in a control tube by a thermometer placed in the milk. The temperature in the tubes was not allowed to vary more than half a degree in either direction. In all experiments the heating period was 30 minutes at a given temperature. After the heating, the tubes of milk were quickly cooled to about 10° C. (50° F.), incubated at 37° C. (98.6° F.), and the reactions recorded after 24, 48, 72, and 96 hours. Growth in the tube indicated that the organism was not destroyed at the particular temperature to which the milk had been subjected. In every case the tubes were run in duplicate, and in general both tubes had to show growth before the test was considered positive. The only exceptions to this were cases in which only one of the tubes showed growth after the highest heating temperature; in such cases one tube was considered a positive reaction and the organism was recorded as surviving the process.

This method of determining the thermal death point was used, in order to render the conditions of heating similar to pasteurization.

THE THERMAL DEATH POINT OF THE CULTURES AS A WHOLE

Studies were made of the thermal death point of 174 cultures of colon bacilli isolated from the following sources: 154 from cow feces, 16 from milk and cream, 2 from flies, 1 from human feces, and 1 from cheese. (The cultures were supplied through the courtesy of Mr. L. A. Rogers, in charge of the research laboratory of the Dairy Division.) All of the organisms would be classified as colon bacilli according to the usual cultural tests for *Bacillus coli*. These cultures, with the exception of two not studied and three noted below, were typical colon bacilli of the

B. coli communis or *communior* type according to the studies of Rogers, Clark, and Davis (7), also Rogers, Clark, and Evans (8). The three cultures not typical were probably of the *B. aerogenes* type. The cultures were heated in milk as previously described to temperatures ranging from 51.7° C. (125° F.) to 68.3° C. (155° F.). The results given in Table I show the number and percentage of cultures which withstood the different temperatures.

TABLE I.—*Effect of heat on colon bacilli—all cultures*

Cultures surviving.	Exposed for 30 minutes at—						
	51.7° C. (125° F.).	54.5° C. (130° F.).	57.2° C. (135° F.).	60° C. (140° F.).	62.8° C. (145° F.).	65.6° C. (150° F.).	68.3° C. (155° F.).
Number	174	173	158	95	12	1	0
Per cent	100. 00	99. 43	90. 80	54. 59	6. 89	0. 57	0

It is seen from the table that 95, or 54.59 per cent of all the cultures, survived at 60° C. (140° F.). This is particularly interesting, since this temperature is the lowest used in commercial pasteurization. When heated to 62.8° C. (145° F.), 12, or 6.89 per cent, of the cultures survived. This temperature of 62.8° C. (145° F.) maintained for 30 minutes is the temperature generally used in the process of pasteurization. Only one culture survived a temperature of 65.6° C. (150° F.), and this culture when heated again failed to survive at this temperature.

These results are shown more clearly in figure 1, where they have been plotted. One of the cultures was destroyed at a temperature as low as 54.5° C. (130° F.). It is interesting to note that at 60° C. (140° F.) 95 of the cultures survived, while at 62.8° C. (145° F.) a difference of only 2.8° C. or 5° F., only 12 survived. In other words, 87.3 per cent of the cultures which survived at 60° C. (140° F.) were destroyed at 62.8° C. (145° F.).

It is very evident from these results that colon bacilli may survive the process of pasteurization when a temperature of 62.8° C. (145° F.) is used.

VARIATION IN THE THERMAL DEATH POINT OF THE CULTURES

In order to determine whether the colon bacilli which survive at 62.8° C. (145° F.) would exhibit the same ability in repeated heatings, the same cultures were reheated to that temperature six times, with the results shown in Table II.

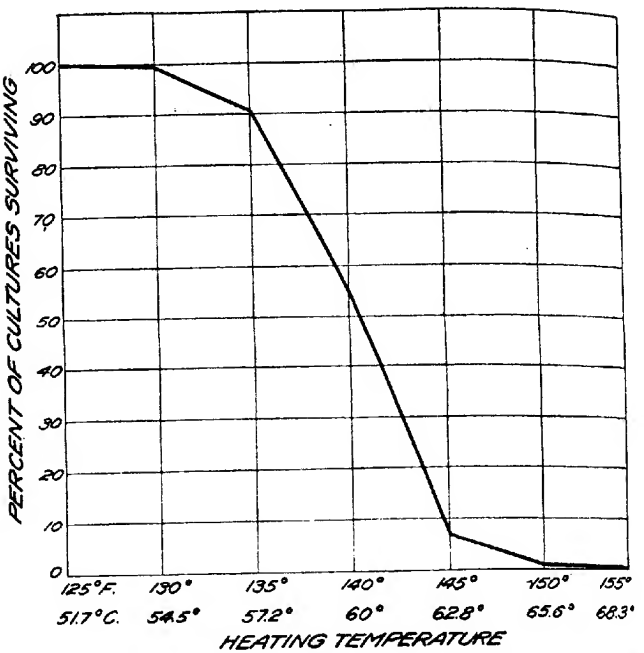


FIG. 1.—Curve showing results of heating cultures of colon bacilli for 30 minutes at various temperatures.

TABLE II.—*Variation in the ability of colon bacilli to survive pasteurization for 30 minutes at 62.8° C. (145° F.)*

Culture No.	Number of heatings.						Summary.		
	First.	Second.	Third.	Fourth.	Fifth.	Sixth.	Both tubes.	Both tubes.	One tube and one tube.
GV	+ +	+ −	− −	+ +	+ +	− −	3	2	
HO	+ −	− −	+ −	− −	+ −	− −	0	0	
HP	+ −	+ −	+ +	+ +	− −	− −	2	2	
IF	+ −	− −	+ −	− −	+ −	− −	0	3	
IJ	+ −	− −	− −	− −	+ −	− −	0	4	
IL	+ −	+ −	+ +	+ −	− −	− −	1	2	
IN	+ −	− −	− −	+ −	+ −	− −	0	3	
IS	+ −	− −	+ −	− −	+ +	− −	1	3	
IY	+ −	− −	+ −	+ −	+ −	− −	0	2	
MF	+ −	− −	− −	− −	+ −	− −	0	4	
MT	+ −	− −	+ −	+ +	+ −	− −	1	2	
NV	+ −	+ −	+ −	− −	− −	− −	0	3	
Total cultures	12	12	12	12	12	12			
Positive	12	4	8	6	9	0			

In the above experiments two litmus-milk tubes were inoculated in the usual way with each of the 12 cultures and were heated for 30 minutes at 62.8° C. (145° F.). The first experiment shows the record of the 12 cultures which survived in the determination of the thermal death point of the cultures as a whole. When these 12 cultures were again heated, only 4 survived, on the third trial 8, on the fourth trial 6, on the fifth trial 9, and on the sixth trial none survived.

These results are important since they show that at 62.8° C. (145° F.) colon bacilli may or may not survive a process of pasteurization. It is evident that this is a critical temperature and that occasionally colon bacilli may survive, owing in all probability to the resistance of a few cells in the culture. This explanation is supported by the figures in the summary of Table II, in which is seen the number of times that both the litmus-milk tubes showed growth; also when both tubes were negative and when one tube was positive and one negative.

It may be seen, also, that the same culture on repeated heating does not give the same results. It is evident that certain strains of colon bacilli have a thermal death point which is close to 62.8° C. (145° F.), and although they represent only a small percentage of the cultures we studied, the fact that such cultures exist complicates the colon test for efficiency of pasteurization.

The apparent scarcity of these resistant colon bacilli and the fact that 62.8° C. (145° F.) is near their thermal death point explains our failure to find them in the samples pasteurized by us under laboratory conditions, as stated previously in this paper.

HEAT RESISTANCE OF COLON BACILLI

From these results it seems that the colon bacilli as a rule have a low majority thermal death point and the cultures survive the higher temperatures only by reason of the resistance of a few cells.

Gage and Stoughton (3) found this to be true of a few cultures which they studied, and although they tried to breed a race with a high majority thermal death point, their efforts were not successful. We have also tried to breed a resistant type, but thus far without success.

In our experiments the thermal death point determinations were made in duplicate tubes of litmus milk, and the appearance of growth was recorded after an incubation period of 24, 48, 72, and 96 hours. In every case control tubes not heated showed a marked acid reaction in 24 hours, but in the heated tubes with the same inoculation the growth was often delayed, so that sometimes no reaction was noticed until after 96 hours, incubation. When the reaction was delayed, it showed that a portion of the bacteria in the milk were destroyed by the heating. When the heating has little or no effect, the heated tubes should show a positive reaction

in 24 hours the same as the control tubes. In Table III we have recorded the percentage of cultures which gave a positive reaction in both of the litmus-milk tubes and in only one milk tube after different periods of incubation and when heated at different temperatures.

TABLE III.—*Effect of heat in relation to the time required for cultures to show growth*

Hours of incubation.	Tube reaction.	54.5° C. (130° F.).	57.2° C. (135° F.).	60° C. (140° F.).	62.8° C. (145° F.).
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
24	2 tubes+	92.75	28.34	11.11	12.50
	1 tube+, 1 tube−	0	14.17	26.98	0
48	2 tubes+	5.80	24.41	17.46	0
	1 tube+, 1 tube−	1.45	12.59	20.63	62.50
72	2 tubes+	0	5.52	3.17	0
	1 tube+, 1 tube−	0	11.03	19.06	25.00
96	2 tubes+	0	0	0	0
	1 tube+, 1 tube−	0	3.94	1.59	0
Total		100.00	100.00	100.00	100.00

From the table it will be seen that 92.75 per cent of the cultures showed a positive reaction after 24 hours' incubation in both of the duplicate tubes when heated at 54.5° C. (130° F.). After 48 hours' incubation 5.8 per cent more of the cultures showed a positive reaction in both tubes, while 1.45 per cent showed a positive reaction in only one of the two tubes. At 62.8° C. (145° F.), however, only a small percentage of the cultures were positive after 24 hours. The majority required from 48 to 72 hours' incubation to show growth. This shows that a large proportion of the cells were destroyed so that a longer incubation was necessary to allow bacterial increases sufficient to cause a positive reaction. These facts are further supported by the differences in the number of cultures in which both duplicate tubes showed a positive reaction. At 62.8° C. (145° F.) only a small percentage of the cultures showed a positive reaction in both tubes, showing that in many cases all the bacteria in one tube were destroyed, while in the duplicate tube a few cells only survived.

It is of interest to note that the colon bacilli are less heat-resistant than the streptococci, as is shown in a previous paper (2).

THE PRESENCE OF COLON BACILLI AS A TEST OF THE EFFICIENCY OF PASTEURIZATION

The growth of colon bacilli which survive pasteurization is a matter of considerable importance, particularly when the presence of *B. coli* in pasteurized milk is considered as an index of the efficiency of the process. We have therefore studied the effect of pasteurization at 62.8° C. (145° F.) on two cultures of colon bacilli which were known to be able to survive heating at that temperature. Flasks of sterile skim milk were inoculated with several cubic centimeters of an 18-hour-old broth culture of a colon bacillus. The number of bacteria in the milk was determined before heating and again after the pasteurized milk had been allowed to stand for 24, 48, and 72 hours at room temperature. The bacteria at the end of the 24-hour period were determined by placing as high as 3 cubic centimeters in large petri plates. Table IV shows the results of an experiment with the two colon cultures, GV and HO. Three flasks of milk were inoculated from each culture with the same amount of broth, but a bacterial count was made on only one of the three flasks before heating.

TABLE IV.—*Growth of Bacillus coli in milk heated for 30 minutes at 62.8° C. (145° F.) and held at room temperature*

Culture No.	Flask.	Number of bacteria per cubic centimeter.			
		Before pasteurization.	After pasteurization.		
			24 hours.	48 hours.	72 hours.
GV	1	5,000,000	0 in 3 c. c.	1,050,000	20,000,000
	2		1 in 3 c. c.	20,000	1,140,000,000
	3		0 in 3 c. c.	210,000,000	1,450,000,000
HO	1	6,000,000	0 in 3 c. c.	61,000,000	1,750,000,000
	2		0 in 2 c. c.	172,000,000	1,250,000,000
	3		1 in 3 c. c.	160,000,000	800,000,000

It may be seen from the table that, although the cultures survived the pasteurization at 62.8° C. (145° F.), there was a very great cell destruction, as the bacterial count after the flasks had stood for 24 hours at room temperature was very low. However, in 48 hours' time there was a very large bacterial increase and even more after 72 hours.

A similar experiment was repeated with the same cultures, except that the milk after heating was held in a refrigerator at 8 C. (46.4° F.). The results in Table V show again the great destruction of bacterial cells which takes place during the heating process. A few bacteria survived, but very little increase took place at the low temperature.

TABLE V.—*Growth of Bacillus coli in milk heated for 30 minutes at 62.8° C. (145° F.) and held at 8° C. (46.4° F.)*

Culture No.	Flask.	Number of bacteria per cubic centimeter.							
		Before pasteurization.	After pasteurization.						
			1 day.	2 days.	3 days.	4th–9th day.	3 weeks.	4 weeks.	6 weeks.
GV.....	1	4,000,000	0 in 3 c.c.	0 in 3 c.c.	0 in 3 c.c.	0 in 3 c.c.	50	10	0 in 3 c.c.
HO.....	1	7,000,000	1 in 3 c.c.	0 in 3 c.c.	0 in 3 c.c.	0 in 3 c.c.	1,000	60	0 in 3 c.c.
	2		1 in 3 c.c.	0 in 3 c.c.	0 in 3 c.c.	0 in 3 c.c.	500	70	0 in 3 c.c.

The relation of these results to commercial pasteurization can be plainly seen. Milk is pasteurized usually at 62.8° C. (145° F.) for 30 minutes and in subsequent handling is kept at various temperatures from low to high, depending on conditions, until it is consumed. In view, therefore, of the results of our experiments, it is possible to explain the presence of colon bacilli in pasteurized milk on the ground of their ability to survive the process.

These results, however, indicate that colon bacilli survive pasteurization on account of the resistance of a few cells and not because the cultures have a high majority thermal death point, in which case a large number of cells would survive. Since it is apparent that colon bacilli have a low majority thermal death point, we should not expect to find large numbers of these bacteria in pasteurized milk immediately after the heating process. If this condition is found we should believe from our results that the presence of the bacilli would indicate inefficient heating or a heavy reinfection.

We must call attention to the fact that these opinions are based on a study of 174 cultures of colon bacilli, and consequently, while they represent a considerable number of strains of *Bacillus coli*, it is possible that a study of still more cultures might yield different results. It is not improbable that colon bacilli with a high majority thermal death point do exist, and if such is the case large numbers might be found immediately after pasteurization.

SUMMARY AND CONCLUSIONS

(1) The thermal death point of 174 cultures of colon bacilli isolated from cow feces, milk and cream, human feces, flies, and cheese showed considerable variation when the cultures were heated in milk for 30 minutes under conditions similar to pasteurization.

At 60° C. (140° F.), the lowest pasteurizing temperature, 95 cultures, or 54.59 per cent, survived; at 62.8° C. (145° F.), the usual temperature for pasteurizing, 12, or 6.89 per cent, survived. One culture was not destroyed at 65.6° C. (150° F.) on the first heating, but in repeated experiments it was always destroyed.

(2) There is a marked difference in the effect of heating at 60° C. (140° F.) and at 62.8° C. (145° F.). Although there is only a difference of 2.8° C., or 5° F., 87.3 per cent of the cultures which survived at 60° C. (140° F.) were destroyed at 62.8° C. (145° F.).

(3) Considerable variation was found in the thermal death point of the colon bacilli which survived 62.8° C. (145° F.). When the 12 cultures which survived were heated again at the same temperature, it was found that many did not survive and in each repeated heating different results were obtained.

It seems evident that 62.8° C. (145° F.) maintained for 30 minutes is a critical temperature for colon bacilli.

(4) Among the 174 cultures studied all were found to have a low majority thermal death point, but were able to survive pasteurizing temperatures on account of the survival of a few cells.

(5) The colon test as an index of the efficiency of the process of pasteurization is complicated by the ability of certain strains to survive a temperature of 62.8° C. (145° F.) for 30 minutes and to develop rapidly when the pasteurized milk is held under temperature conditions which might be met during storage and delivery.

The presence of a large number of colon bacilli immediately after the heating process may indicate improper treatment of the milk.

(6) If milk is pasteurized at a temperature of 65.6° C. (150° F.) or above for 30 minutes, we should not expect, from our results, that any colon bacilli would survive. Consequently under such conditions the colon test for the efficiency of pasteurization may be of value. It must be remembered, however, that a study of more cultures may reveal strains of colon bacilli that are able to survive this and even higher temperatures.

LITERATURE CITED

(1) AYERS, S. H., and JOHNSON, W. T., Jr.
1913. A study of the bacteria which survive pasteurization. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 161, 66 p., 30 fig.

(2) ——— ———
1914. Ability of streptococci to survive pasteurization. *In* Jour. Agr. Research, v. 2, no. 4, p. 321–330, 3 fig.

(3) GAGE, S. DE M., and STOUGHTON, GRACE VAN E.
1906. A study of the laws governing the resistance of Bacillus coli to heat. *In* Technol. Quart., v. 19, no. 1, p. 41–54.

(4) GEUNS, JB. VAN.
1889. Ueber das "Pasteurisiren" von Bacterien. Ein Beitrag zur Biologie der Mikroorganismen. *In* Arch. Hyg., Bd. 9, p. 369–402.

(5) JONG, D. A. DE, and GRAAFF, W. C. DE.
1906. Die Coli-Kontrolle der pasteurisierten Milch. *In* Nederland. Weekbl. Zuivelber. Veeteelt, deel 12, afd. 37–38. *Abstract in* Milchw. Zentralbl., Jahrg. 3, Heft 6, p. 265–268. 1907. Original not seen.

(6) RINGELING, H. G.
1903. Beitrag zur bakteriologischen Kontrolle sog. krankheitskeimfreier pasteurisierter und hochpasteurisierter Milch. *In* Milch Ztg., Jahrg. 32, no. 52, p. 818–819. Tr. from the Dutch.

(7) ROGERS, L. A., CLARK, W. M., and DAVIS, B. J.
1914. The colon group of bacteria. *In* Jour. Infect. Diseases, v. 14, no. 3, p. 411–475.

(8) ——— ——— and EVANS, ALICE C.
1914. The characteristics of bacteria of the colon type found in bovine feces. *In* Jour. Infect. Diseases, v. 15, no. 1, p. 99–123

(9) ZELENSKI, THADDÆUS.
1906. Zur Frage der Pasteurisation der Säuglingsmilch. *In* Jahrb. Kinderheilk., Bd. 63, p. 288–307. *Abstract in* Centralbl. Bakt. [etc.], Abt. 2, Bd. 18, no. 4/6, p. 175. 1907. Original not seen.

PRELIMINARY AND MINOR PAPERS

FITTING LOGARITHMIC CURVES BY THE METHOD OF MOMENTS [1]

By JOHN RICE MINER,

Computer, Maine Agricultural Experiment Station

WITH AN INTRODUCTORY STATEMENT ON THE USE OF LOGARITHMIC CURVES IN BIOLOGICAL AND AGRICULTURAL INVESTIGATIONS BY RAYMOND PEARL, BIOLOGIST, MAINE AGRICULTURAL EXPERIMENT STATION

INTRODUCTORY STATEMENT

The use of logarithmic curves in the analysis of various kinds of biological and agricultural data is rapidly becoming widespread and general. It was first shown by Lewenz and Pearson (13, 22) [2] that the growth of children followed a logarithmic curve. The present writer (17) demonstrated that the phenomena of growth and differentiation in Ceratophyllum also followed a logarithmic curve. Donaldson (2, 3, 4, 5, 6) and Hatai (8, 9, 10) in a series of papers dealing with the growth and quantitative relations of the whole organism and its various parts in the white rat and the frog have shown that the same law holds for growth in those forms.

Other biological phenomena than growth follow a logarithmic law. Pearl (14), in a case of regulation of the shape of abnormal eggs, and later Curtis (1) for normal eggs, have shown that the changes in size and shape of successively laid eggs are graduated with a logarithmic curve. Work now in progress in the Biological Laboratory, Maine Experiment Station, of which only a preliminary notice has yet been published (15), shows that generally the change in milk flow with age in dairy cattle is logarithmic. Several years ago Holtsmark (12) pointed out that the relation between the number of food units required and the milk yields of different animals was logarithmic.

From this incomplete review of the literature recording the use of logarithmic curves in biological and agricultural investigations it is clear that the workers in these fields will, as time goes on, have increasing need to be able to handle these curves easily and critically.

Up to the present time the only available method of fitting logarithmic curves was that of least squares. Several years ago Pearl and McPheters (16) published a set of tables intended to lighten materially the labor of fitting such curves by the least-squares method. For a long time, however, the writer has felt that it would be highly desirable to bring this class of curves into the general system of curve fitting worked out by Pearson (18, 19, 20, 21, 23), and known as the "method of moments." The theory of the method is extremely simple, involving as

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 78.

[2] Reference is made by number to "Literature cited," p. 422.

it does only the assumption that if we equate the area and moments of a theoretical curve to the area and moments of a series of observations we shall get a reasonable fit of the curve to the observations. Experience with the method in the hands of different workers in England and America has abundantly demonstrated that this assumption is entirely justified in the fact.

In the papers cited, and in others also, Pearson has given the equations for the calculation of the constants from the moments in the case of (*a*) skew frequency curves in general, (*b*) sine curves, (*c*) parabolas of all orders, (*d*) the point binomial, (*e*) hypergeometrical series, etc. There has been lacking, however, the determination of the equations connecting moments and constants for the general family of logarithmic curves of the type

$$y=a+bx+cx^2+d\log(x+\alpha)$$

and its modifications. I suggested some time ago to Mr. Miner that he attack the problem, which, while theoretically simple and straightforward, proved rather laborious in the actual carrying out. This he has done, with the results set forth in this paper.

RAYMOND PEARL

MOMENTS OF A LOGARITHMIC CURVE

GENERAL CASE

Let $y_1, y_2, y_3, \ldots, y_k, \ldots, y_m$ be a series of ordinates with corresponding abscissæ $x_1, x_2, x_3, \ldots, x_k, \ldots x_m$ to which is to be fitted the curve $y=a+bx+c\log_{10}x$.

Let the unit of calculation $= x_2-x_1$ and the origin be placed at $2x_1-x_2$. The abscissæ expressed in units of calculation and taken from the new origin will then be $1, 2, x'_3, \ldots, x'_k, \ldots x'_m$. In calculating the moments each ordinate must first be multiplied by the base, $h_{x'_k}$, of the rectangle of which it is the mid line. Otherwise the moments will represent not the whole area, but strips of unit base of which the ordinates are the mid lines. For the first three ordinates $h_{x'_k}$ is $1, 1, 2x'_3-5$, respectively; for higher ordinates it is found from the equation $h_{x'_k}=2x'_k-4x'_{k-1}+4x'_{k-2}-\ldots-(-1)^k 4x'_3+(-1)^k 5$. The upper limit of the area is given by $2x'_m-2x'_{m-1}+\ldots-(-1)^k 2x'_3+(-1)^k \frac{5}{2}=l+q$, where l is the integral and q the fractional portion of the number.

Let M_n represent the nth moment about the origin as above chosen. Then

$$M_n=\int_{\frac{1}{2}}^{l+q}(a+bx+c\log_{10}x)\,x^n dx=\frac{a}{n+1}\left[(l+q)^{n+1}-\left(\frac{1}{2}\right)^{n+1}\right]$$

$$+\frac{b}{n+2}\left[(l+q)^{n+2}-\left(\frac{1}{2}\right)^{n+2}\right] \qquad \text{(i)}$$

$$+c\log_{10}e\left[(l+q)^{n+1}\left\{\frac{\log_e(l+q)}{n+1}-\frac{1}{(n+1)^2}\right\}-\left(\frac{1}{2}\right)^{n+1}\left\{\frac{\log_e\frac{1}{2}}{n+1}-\frac{1}{(n+1)^2}\right\}\right]$$

Putting $n=0, 1, 2$, successively, we obtain the three equations which being solved give us the constants of the curve:

$$M_0=a\left(l+q-\frac{1}{2}\right)+\frac{b}{2}\left[(l+q)^2-\frac{1}{4}\right]+c\log_{10}e\left[(l+q)\log_e(l+q)-\frac{1}{2}\log_e\frac{1}{2}+\frac{1}{2}-(l+q)\right] \quad \text{(ii)}$$

$$M_1=\frac{a}{2}\left[(l+q)^2-\frac{1}{4}\right]+\frac{b}{3}\left[(l+q)^3-\frac{1}{8}\right]+\frac{c}{2}\log_{10}e\left[(l+q)^2\log_e(l+q)-\frac{1}{4}\log_e\frac{1}{2}+\frac{1}{8}-\frac{1}{2}(l+q)^2\right] \quad \text{(iii)}$$

$$M_2=\frac{a}{3}\left[(l+q)^3-\frac{1}{8}\right]+\frac{b}{4}\left[(l+q)^4-\frac{1}{16}\right]+\frac{c}{3}\log_{10}e\left[(l+q)^3\log_e(l+q)-\frac{1}{8}\log_e\frac{1}{2}+\frac{1}{24}-\frac{1}{3}(l+q)^3\right] \quad \text{(iv)}$$

Multiplying equation ii by $\frac{1}{2}\left(l+q+\frac{1}{2}\right)$ and by $\frac{1}{3}\left[(l+q)^2+\frac{1}{2}(l+q)+\frac{1}{4}\right]$ and subtracting from equation iii and iv, respectively:

$$2M_1-\left(l+q+\frac{1}{2}\right)M_0=\frac{b}{6}\left(l+q-\frac{1}{2}\right)^3+\frac{c}{2}\log_{10}e\left[(l+q)\left\{\log_e\frac{1}{2}-\log_e(l+q)\right\}+(l+q)^2-\frac{1}{4}\right] \quad \text{(v)}$$

$$3M_2-\left[(l+q)^2+\frac{1}{2}(l+q)+\frac{1}{4}\right]M_0=\frac{b}{4}\left(l+q+\frac{1}{2}\right)\left(l+q-\frac{1}{2}\right)^3+\frac{c}{2}\log_{10}e\left[(l+q)\left(l+q+\frac{1}{2}\right)\left\{\log_e\frac{1}{2}-\log_e(l+q)\right\}+\frac{4}{3}(l+q)^3-\frac{1}{6}\right] \quad \text{(vi)}$$

Multiplying equation v by $\frac{3}{2}\left(l+q+\frac{1}{2}\right)$ and subtracting from equation vi:

$$6\left[M_2-\left(l+q+\frac{1}{2}\right)M_1\right]+\left[(l+q)^2+2(l+q)+\frac{1}{4}\right]M_0=\frac{c}{2}\log_{10}e\left[(l+q)\left(l+q+\frac{1}{2}\right)\left\{\log_e(l+q)-\log_e\frac{1}{2}\right\}-\frac{1}{3}\left\{(l+q)^3+\frac{9}{2}(l+q)^2-\frac{9}{4}(l+q)-\frac{1}{8}\right\}\right] \quad \text{(vii)}$$

$$c=\frac{6\left[\left(l+q+\frac{1}{2}\right)M_1-M_2\right]-\left[(l+q)^2+2(l+q)+\frac{1}{4}\right]M_0}{\frac{1}{2}\left[(l+q)\left(l+q+\frac{1}{2}\right)\left\{\log_{10}\frac{1}{2}-\log_{10}(l+q)\right\}+\frac{1}{3}\left(l+q-\frac{1}{2}\right)\left\{(l+q)^2+5(l+q)+\frac{1}{4}\right\}\log_{10}e\right]} \quad \text{(viii)}$$

$$b=\frac{6}{\left(l+q-\frac{1}{2}\right)^3}\left[2M_1-\left(l+q+\frac{1}{2}\right)M_0 - \frac{c}{2}\left\{(l+q)\left(\log_{10}\frac{1}{2}-\log_{10}\overline{l+q}\right)+\left(\overline{l+q}^2-\frac{1}{4}\right)\log_{10}e\right\}\right] \tag{ix}$$

$$a=\frac{1}{l+q-\frac{1}{2}}\left[M_0-\frac{b}{2}\left\{(l+q)^2-\frac{1}{4}\right\}-c\left\{(l+q)\log_{10}(l+q) -\frac{1}{2}\log_{10}\frac{1}{2}-\left(l+q-\frac{1}{2}\right)\log_{10}e\right\}\right] \tag{x}$$

SPECIAL CASES

In the preceding section the simplest form of logarithmic curve in practical biometric use is considered. Extended experience in the Biological Laboratory of the Maine Experiment Station has shown that this simple form of the curve is only rarely adequate in the fitting of biological data. Usually one or the other, or both, of two modifications is found to be necessary before a suitable logarithmic curve is found. One of these, first used in biometric work by Pearl (17), in his studies of the growth of Ceratophyllum, later by Hatai (8) and others, is to add another constant, α, so that the equation then becomes

$$y=a+bx+c\log(x+\alpha).$$

The second modification is made by adding a term in x^2 to the equation. This modification is necessary in the wide range of cases where, after reaching a maximum, the values of the ordinates decrease with increasing values of x. This curve with the x^2 term was first used by Pearl to describe the change in shape of successively laid eggs of a particular hen (14). A logarithmic curve of this type

$$y=a+bx+cx^2+d\log x$$

appears, from rather extensive experience in this laboratory, to be the general form of expression of the quantitative changes in an organism throughout its life—that is, *including both growth and senescence*.

The equations for determining the constants of the curve

$$y=a+bx+cx^2+d\log_{10}x$$

are as follows:

$$d=\frac{\left\{\begin{array}{c}20M_3-30\left(l+q+\frac{1}{2}\right)M_2+3[4(l+q)^2+6(l+q)+1]M_1 \\ -\left(l+q+\frac{1}{2}\right)\left[(l+q)^2+4(l+q)+\frac{1}{4}\right]M_0\end{array}\right\}}{\left\{\begin{array}{l}\frac{1}{2}\left[(l+q)\left\{(l+q)^2+\frac{3}{2}(l+q)+\frac{1}{4}\right\}\left\{\log_{10}\frac{1}{2}-\log_{10}(l+q)\right\}\right. \\ \left.+\frac{1}{6}\left\{(l+q)^2-\frac{1}{4}\right\}\left\{(l+q)^2+14(l+q)+\frac{1}{4}\right\}\log_{10}e\right]\end{array}\right\}} \tag{xi}$$

$$c=\frac{30}{\left(l+q-\frac{1}{2}\right)^5}\left[6\left\{M_2-\left(l+q+\frac{1}{2}\right)M_1\right\}+\left\{(l+q)^2+2(l+q)+\frac{1}{4}\right\}M_0 -\frac{d}{2}\left\{(l+q)\left(l+q+\frac{1}{2}\right)\left(\log_{10}\overline{l+q}-\log_{10}\frac{1}{2}\right) -\frac{1}{3}\left(\overline{l+q}^3+\frac{9}{2}\overline{l+q}^2-\frac{9}{4}\overline{l+q}-\frac{1}{8}\right)\log_{10}e\right\}\right] \tag{xii}$$

$$b=\frac{6}{\left(l+q-\frac{1}{2}\right)^3}\left[2M_1-\left(l+q+\frac{1}{2}\right)M_0-\frac{c}{6}\left(l+q+\frac{1}{2}\right)\left(l+q-\frac{1}{2}\right)^3 -\frac{d}{2}\left\{(l+q)\left(\log_{10}\frac{1}{2}-\log_{10}\overline{l+q}\right)+\left(\overline{l+q}^2-\frac{1}{4}\right)\log_{10}e\right\}\right] \tag{xiii}$$

$$a=\frac{1}{l+q-\frac{1}{2}}\left[M_0-\frac{b}{2}\left\{\left(l+q\right)^2-\frac{1}{4}\right\}-\frac{c}{3}\left\{\left(l+q\right)^3-\frac{1}{8}\right\} -d\left\{(l+q)\log_{10}(l+q)-\frac{1}{2}\log_{10}\frac{1}{2}-\left(l+q-\frac{1}{2}\right)\log_{10}e\right\}\right] \tag{xiv}$$

For the curve

$$y=a+bx+c\log_{10}(x+\alpha)$$

the equations for determining the constants are:

$$c=\frac{6\left[\left(l+q+\frac{1}{2}\right)M_1-M_2\right]-\left[(l+q)^2+2(l+q)+\frac{1}{4}\right]M_0}{\left\{\left(\alpha+\frac{1}{2}\right)(l+q+\alpha)\left(l+q+2\alpha+\frac{1}{2}\right)\left[\log_{10}\left(\alpha+\frac{1}{2}\right)-\log_{10}(l+q+\alpha)\right] +\left(l+q-\frac{1}{2}\right)\left[\frac{1}{6}\left\{(l+q)^2+5(l+q)+\frac{1}{4}\right\}+2\alpha\left(l+q+\frac{1}{2}\right)+2\alpha^2\right]\log_{10}e\right\}} \tag{xv}$$

$$b=\frac{6}{\left(l+q-\frac{1}{2}\right)^3}\left[2M_1-\left(l+q+\frac{1}{2}\right)M_0-c\left\{\left(\alpha+\frac{1}{2}\right)(l+q+\alpha)\left(\log_{10}\overline{\alpha+\frac{1}{2}} -\log_{10}\overline{l+q+\alpha}\right)+\frac{1}{2}\left(l+q-\frac{1}{2}\right)\left(l+q+2\alpha+\frac{1}{2}\right)\log_{10}e\right\}\right] \tag{xvi}$$

$$a=\frac{1}{l+q-\frac{1}{2}}\left[M_0-\frac{b}{2}\left\{(l+q)^2-\frac{1}{4}\right\}-c\left\{(l+q+\alpha)\log_{10}(l+q+\alpha) -\left(\alpha+\frac{1}{2}\right)\log_{10}\left(\alpha+\frac{1}{2}\right)-\left(l+q-\frac{1}{2}\right)\log_{10}e\right\}\right] \tag{xvii}$$

By the use of the third moment an equation might also be derived for determining α. As this, however, is a somewhat complex logarithmic expression, from which α can be obtained only after much labor, it has seemed best to determine α empirically, as in working by the method of least squares.

ORDINATES EQUALLY DISTRIBUTED

Up to this point we have considered that the ordinates were distributed at any irregular points on the base line. In the usual case, however, they will, of course, be at equal intervals from one another. When the ordinates are given at equal intervals, i. e., when $x_2-x_1=x_3-x_2=\cdots=x_m-x_{m-1}$, l becomes equal to the number of given ordinates, $q=\frac{1}{2}$, and the equations for the constants can be put in a somewhat simpler form.

For the curve $y=a+bx+c\log_{10}x$:

$$c=j_1[6\{(l+1)M_1-M_2\}-(l^2+3l+1.5)M_0] \qquad \text{(xviii)}$$

$$b=\frac{6}{l^3}[2M_1-(l+1)M_0-j_2c] \qquad \text{(xix)}$$

$$a=\frac{1}{l}M_0-\frac{l+1}{2}b-j_3c \qquad \text{(xx)}$$

where

$$j_1=2\left[(l+1)\left(l+\tfrac{1}{2}\right)\left\{\log_{10}\tfrac{1}{2}-\log_{10}\left(l+\tfrac{1}{2}\right)\right\}+\frac{l}{3}(l^2+6l+3)\log_{10}e\right]^{-1}$$

$$j_2=\frac{3}{l^3}\left[\left(l+\tfrac{1}{2}\right)\left\{\log_{10}\tfrac{1}{2}-\log_{10}\left(l+\tfrac{1}{2}\right)\right\}+l(l+1)\log_{10}e\right]$$

$$j_3=\frac{1}{l}\left[\left(l+\tfrac{1}{2}\right)\log_{10}\left(l+\tfrac{1}{2}\right)-\tfrac{1}{2}\log_{10}\tfrac{1}{2}-l\log_{10}e\right]$$

For the curve $y=a+bx+cx^2+d\log_{10}x$:

$$d=j_4[20M_3-30(l+1)M_2+j_5M_1-j_6M_0] \qquad \text{(xxi)}$$

$$c=\frac{30}{l^5}[6\{M_2-(l+1)M_1\}+(l^2+3l+1.5)M_0]+j_7d \qquad \text{(xxii)}$$

$$b=\frac{6}{l^3}[2M_1-(l+1)M_0]-(l+1)c-j_4d \qquad \text{(xxiii)}$$

$$a=\frac{1}{l}M_0-\frac{l+1}{2}b-j_6c-j_5d \qquad \text{(xxiv)}$$

where

$$j_4=2\left[\left(l+\tfrac{1}{2}\right)\left(l^2+\tfrac{5}{2}l+\tfrac{5}{4}\right)\left\{\log_{10}\tfrac{1}{2}-\log_{10}\left(l+\tfrac{1}{2}\right)\right\}+\frac{l}{6}(l+1)\left(l^2+15l+\tfrac{15}{2}\right)\log_{10}e\right]^{-1}$$

$$j_5=12\left(l^2+\tfrac{5}{2}l+\tfrac{5}{4}\right)$$

$$j_6=(l+1)\left(l^2+5l+\tfrac{5}{2}\right)$$

$$j_7=\frac{30}{l^5}\left[\tfrac{1}{2}(l+1)\left(l+\tfrac{1}{2}\right)\left\{\log_{10}\tfrac{1}{2}-\log_{10}\left(l+\tfrac{1}{2}\right)\right\}+\frac{l}{6}(l^2+6l+3)\log_{10}e\right]$$

$$j_8=\frac{1}{3}\left(l^2+\tfrac{3}{2}l+\tfrac{3}{4}\right)$$

From the foregoing it is evident that since the j's involve only l, and with equal intervals for the ordinates l will always be some integer, the values of the j's for a series of values of l can be tabled once for all, and in this way a great deal of labor saved in the ordinary fitting of logarithmic curves. Accordingly tables of the j's and of $l^2+3l+1.5$, $\frac{30}{l^5}$, and $\frac{6}{l^3}$ have been prepared and are given in an appendix at the end of the paper.

For the curve $y=a+bx+c\log_{10}(x+\alpha)$:

$$c=\frac{6[M_2-(l+1)M_1]+(l^2+3l+1.5)M_0}{\alpha'(l+2\alpha')(l+\alpha')\{\log_{10}(l+\alpha')-\log_{10}\alpha'\}-\frac{l}{6}\{l^2+12\alpha' l+12\alpha'^2\}\log_{10}e} \quad \text{(xxv)}$$

$$b=\frac{6}{l^3}\left[2M_1-(l+1)M_0-c\left\{\alpha'(l+\alpha')(\log_{10}\alpha'-\log_{10}\overline{l+\alpha'})+\left(\frac{l^2}{2}+\alpha' l\right)\log_{10}e\right\}\right] \quad \text{(xxvi)}$$

$$a=\frac{1}{l}M_0-\frac{l+1}{2}b-c\cdot\frac{1}{l}[(l+\alpha')\log_{10}(l+\alpha')-\alpha'\log_{10}\alpha'-l\log_{10}e] \quad \text{(xxvii)}$$

where $\alpha'=\alpha+\frac{1}{2}$.

ILLUSTRATIONS OF THE USE OF LOGARITHMIC EQUATIONS

In order to make clear the use of the above equations, some numerical illustrations will be given.

Let us first consider the data contained in Table I. These give the mean milk production in pounds over a 7-day period of Holstein-Friesian cattle at different ages. The data are taken from the official 7-day A. R. O. record of the Holstein-Friesian Association (11). The laborious task of extracting and tabulating these records and calculating the means was carried through by Mr. John W. Gowen, with the assistance of Mr. S. W. Patterson and Miss Anna B. Perkins, all of the Maine Experiment Station. In future publications from this laboratory these figures will be further dealt with, but here they are used solely for purposes of illustrating the method.

TABLE I.—*Mean 7-day milk production of Holstein-Friesian cows at different ages*

Age.	Number of cows.	Mean production.	Age.	Number of cows.	Mean production.
		Pounds.			*Pounds.*
1 yr. 6 mo. to 1 yr. 11 mo.	1,095	290.6	8 yr. to 8 yr. 5 mo.	434	467.0
2 yr. to 2 yr. 5 mo.	3,693	316.7	8 yr. 6 mo. to 8 yr. 11 mo.	432	466.6
2 yr. 6 mo. to 2 yr. 11 mo.	2,330	347.0	9 yr. to 9 yr. 5 mo.	249	464.6
3 yr. to 3 yr. 5 mo.	2,041	376.0	9 yr. 6 mo. to 9 yr. 11 mo.	243	462.5
3 yr. 6 mo. to 3 yr. 11 mo.	1,950	402.9	10 yr. to 10 yr. 5 mo.	149	462.7
4 yr. to 4 yr. 5 mo.	1,627	428.2	10 yr. 6 mo. to 10 yr. 11 mo.	137	460.2
4 yr. 6 mo. to 4 yr. 11 mo.	1,565	447.1	11 yr. to 11 yr. 5 mo.	72	455.2
5 yr. to 5 yr. 5 mo.	1,195	457.2	11 yr. 6 mo. to 11 yr. 11 mo.	67	453.7
5 yr. 6 mo. to 5 yr. 11 mo.	1,142	464.2	12 yr. to 12 yr. 5 mo.	37	449.1
6 yr. to 6 yr. 5 mo.	882	466.3	12 yr. 6 mo. to 12 yr. 11 mo.	35	444.0
6 yr. 6 mo. to 6 yr. 11 mo.	850	468.0	13 yr. to 13 yr. 5 mo.	20	443.2
7 yr. to 7 yr. 5 mo.	685	466.6	13 yr. 6 mo. to 13 yr. 11 mo.	22	448.7
7 yr. 6 mo. to 7 yr. 11 mo.	597	466.6	14 yr. to 14 yr. 5 mo.	10	440.0

The problem now is to fit by the method of moments a logarithmic curve of the form

$$y=a+bx+cx^2+d\log x$$

to these milk production means.

The calculations to obtain the moments are given in Table II.

TABLE II.—*Calculation of moments for data on Holstein-Friesian cows in original form*

Age.	y	y'	x'	$y'x'$	$y'x'^2$	$y'x'^3$
1 yr. 6 mo. to 1 yr. 11 mo.	299.6	326.06732	1	326.06732	326.06732	326.06732
2 yr. to 2 yr. 5 mo.	316.7	240.32913	2	480.65826	961.31652	1,922.63304
2 yr. 6 mo. to 2 yr. 11 mo.	347.0	401.76094	3	1,205.28282	3,615.84846	10,847.54538
3 yr. to 3 yr. 5 mo.	376.0	361.44305	4	1,445.77220	5,783.08880	23,132.35520
3 yr. 6 mo. to 3 yr. 11 mo.	407.9	407.9	5	2,039.5	10,197.5	50,987.5
4 yr. to 4 yr. 5 mo.	428.2	428.2	6	2,569.2	15,415.2	92,491.2
4 yr. 6 mo. to 4 yr. 11 mo.	447.1	447.1	7	3,129.7	21,907.9	153,355.3
5 yr. to 5 yr. 5 mo.	457.2	457.2	8	3,657.6	29,260.8	234,086.4
5 yr. 6 mo. to 5 yr. 11 mo.	464.2	464.2	9	4,177.8	37,600.2	338,401.8
6 yr. to 6 yr. 5 mo.	466.3	466.3	10	4,663.0	46,630.0	466,300.0
6 yr. 6 mo. to 6 yr. 11 mo.	468.0	468.0	11	5,148.0	56,628.0	622,908.0
7 yr. to 7 yr. 5 mo.	466.6	466.6	12	5,599.2	67,190.4	806,284.8
7 yr. 6 mo. to 7 yr. 11 mo.	466.6	466.6	13	6,065.8	78,855.4	1,025,120.2
8 yr. to 8 yr. 5 mo.	467.0	467.0	14	6,538.0	91,532.0	1,281,448.0
8 yr. 6 mo. to 8 yr. 11 mo.	466.6	466.6	15	6,999.0	104,985.0	1,574,775.0
9 yr. to 9 yr. 5 mo.	464.6	464.6	16	7,433.6	118,937.6	1,903,001.6
9 yr. 6 mo. to 9 yr. 11 mo.	462.5	462.5	17	7,862.5	133,662.5	2,272,262.5
10 yr. to 10 yr. 5 mo.	460.7	460.7	18	8,292.6	149,266.8	2,686,802.4
10 yr. 6 mo. to 10 yr. 11 mo.	460.2	460.2	19	8,743.8	166,132.2	3,156,511.8
11 yr. to 11 yr. 5 mo.	455.2	455.2	20	9,104.0	182,080.0	3,641,600.0
11 yr. 6 mo. to 11 yr. 11 mo.	453.7	453.7	21	9,527.7	200,081.7	4,201,715.7
12 yr. to 12 yr. 5 mo.	449.1	449.1	22	9,880.2	217,364.4	4,782,016.8
12 yr. 6 mo. to 12 yr. 11 mo.	444.0	426.81041	23	9,816.63943	225,782.70689	5,193,002.25847
13 yr. to 13 yr. 5 mo.	443.2	513.14250	24	12,315.42000	295,570.08000	7,093,681.92000
13 yr. 6 mo. to 13 yr. 11 mo.	448.7	340.49788	25	8,512.44700	212,811.17500	5,320,279.37500
14 yr. to 14 yr. 5 mo.	440.0	493.70138	26	12,836.23588	333,742.13288	8,677,295.45488
Total		11,315.45261		158,369.72291	2,806,320.01587	55,610,556.60929

For reasons which need not be considered here it is usually desirable to use corrected rather than raw values of the moments. Here we have used one of Elderton's (7) correction methods. The column headed y' is obtained from the y column by Elderton's formula V^2—i. e., by multiplying the first and last ordinates by 1.1220486, the second and last but one by 0.7588542, the third and last but two by 1.1578125, and the fourth and last but three by 0.9612847.

From this table we have at once $M_0 = 11,315.45261$; $M_1 = 158,369.72291$; $M_2 = 2,806,320.01587$; $M_3 = 55,610,556.60929$; $l = 26$.

Substituting these values in the equations xxi to xxiv for the curve $y = a + bx + cx^2 + d \log x$, the following values for the constants are found:

$d = 0.000096940\ (20M_3 - 810M_2 + 8907M_1 - 21829.5M_0) = 259.83317$.

$c = 0.000025250\ (6M_2 - 162M_1 + 755.5M_0) + 0.0024102d = -0.0533$.

$b = 0.00034137\ (2M_1 - 27M_0) - 27c - 0.044239d = -6.225$.

$a = 0.0384615M_0 - 13.5b - 238.583333c - 1.0221111d = 266.38$.

The final equation then becomes

$$y = 266.38 - 6.225x - 0.0533x^2 + 259.833 \log_{10} x.$$

The ordinates calculated from this equation in comparison with the observations are given in Table IV.

Before proceeding to any discussion of the fit, let us consider a second example, where the ordinates are at irregular intervals. The data here taken for illustration are the same as those of the preceding example, except that certain of the observations have been arbitrarily combined and the new values so obtained taken as ordinates. Table III shows

[1] It is to be noted that the coefficients as given by Elderton (7, p. 27) are incorrect in the last figure.

how this combination has been carried out, and also the calculation of the moments from ordinates at irregular intervals. Since in this instance the four ordinates at each end are regularly spaced, Elderton's formula V may be used. When the end ordinates are not regularly spaced, this is not applicable, and the ordinates may without serious loss of accuracy be left unmodified.

TABLE III.—*Calculation of moments for data on Holstein-Friesian cows, with grouping of certain ordinates*

Age.	y	y'	$h_{x'_k}$	$h_{x'_k}y'$	x'	$h_{x'_k}y'x'$	$h_{x'_k}y'x'^2$	$h_{x'_k}y'x'^3$
1 yr. 6 mo. to 1 yr. 11 mo...	290. 6	326. 07	1	326. 07	1	326. 07	326. 07	326. 07
2 yr. 6 to 2 yr. 5 mo.....	316. 7	240. 33	1	240. 33	2	480. 66	961. 32	1, 922. 63
2 yr. 6 mo. to 2 yr. 11 mo...	347. 0	401. 76	1	401. 76	3	1, 205. 28	3, 615. 85	10, 847. 55
3 yr. to 3 yr. 5 mo.....	376. 0	361. 44	1	361. 44	4	1, 445. 77	5, 783. 09	23, 132. 35
3 yr. 5 mo. to 3 yr. 11 mo...	407. 9	407. 9	1	407. 9	5	2, 039. 5	10, 197. 5	50, 987. 5
4 yr. to 4 yr. 5 mo.....	428. 2	428. 2	1	428. 2	6	2, 569. 2	15, 415. 2	92, 491. 2
4 yr. 6 mo. to 5 yr. 5 mo....	452. 1	452. 1	2	904. 3	7. 5	6, 782. 25	50, 866. 88	381, 501. 56
5 yr. 6 mo. to 6 yr. 11 mo...	466. 2	466. 2	3	1, 398. 5	10	13, 985. 0	139, 850. 0	1, 398, 500. 0
7 yr. to 7 yr. 11 mo.....	466. 6	466. 6	2	933. 2	12. 5	11, 665. 0	145, 812. 5	1, 822, 656. 25
8 yr. to 9 yr. 5 mo.....	466. 1	466. 1	3	1, 398. 2	15	20, 973. 0	314, 595. 0	4, 718, 925. 0
9 yr. 6 mo. to 9 yr. 11 mo...	462. 5	462. 5	1	462. 5	17	7, 862. 5	133, 662. 5	2, 272, 262. 5
10 yr. to 10 yr. 5 mo.....	460. 7	460. 7	1	460. 7	18	8, 292. 6	149, 266. 8	2, 686, 802. 4
10 yr. 6 mo. to 11 yr. 11 mo.	456. 4	456. 4	3	1, 369. 1	20	27, 382. 0	547, 640. 0	10, 952, 800. 0
12 yr. to 12 yr. 5 mo.....	449. 1	449. 1	1	449. 1	22	9, 880. 2	217, 364. 4	4, 782, 016. 8
12 yr. 6 mo. to 12 yr. 11 mo.	444. 0	426. 81	1	426. 81	23	9, 816. 64	225, 782. 70	5, 193, 002. 26
13 yr. to 13 yr. 5 mo.....	443. 2	513. 14	1	513. 14	24	12, 315. 47	295, 570. 08	7, 093, 681. 92
13 yr. 6 mo. to 13 yr. 11 mo.	448. 7	340. 50	1	340. 50	25	8, 512. 45	212, 811. 17	5, 320, 279. 37
14 yr. to 14 yr. 5 mo.....	440. 0	493. 70	1	493. 70	26	12, 836. 23	333. 742. 13	8. 677. 295. 45
Total.....	.....	.....	.....	11, 315. 45	.....	158. 369. 77	2, 803. 263. 19	55. 479. 430. 81

From Table III we have the following values: $M_0 = 11{,}315.45$; $M_1 = 158{,}369.77$; $M_2 = 2{,}803{,}263.19$; $M_3 = 55{,}479{,}430.81$; $l + q = 26.5$; $l = 26$; $q = 0.5$.

Substituting these values in the equations xi to xiv, we obtain:

$d = 0.000096940\ (20M_3 - 810M_2 + 8907M_1 - 21{,}829.5M_0) = 245.67899.$

$c = 0.000025250\ (6M_2 - 162M_1 + 755.5M_0) + 0.0024102d = -0.1338.$

$b = 0.00034137\ (2M_1 - 27M_0) - 27c - 0.044239d = -3.425.$

$a = 0.0384615M_0 - 13.5b - 238.583333c - 1.022111d = 262.26.$

The final equation then becomes—

$$y = 262.26 - 3.425x - 0.1338x^2 + 245.679 \log_{10} x.$$

Table IV compares the fit of the two curves calculated by the method of moments with that of the curve fitted by the method of least squares. It is apparent that the two methods give results of substantially the same accuracy. By the method of moments the root mean-square error is greater, and the mean error less than by that of least squares. Neither difference is, however, large.

TABLE IV.—*Comparison of the graduation of data on Holstein-Friesian cows by different methods*

	By least squares.				By moments.					
					Ordinates at equal intervals.			Ordinates at unequal intervals.		
	y from data.	*y* from curve.	Δ	Δ^2	*y* from curve.	Δ	Δ^2	*y* from curve.	Δ	Δ^2
1	290.6	272.1	+18.5	342.25	260.1	+30.5	930.25	258.7	+31.9	1,017.61
2	316.7	332.2	−15.5	240.25	331.9	−15.2	231.04	328.8	−12.1	146.41
3	347.0	366.9	−19.9	396.01	371.2	−24.2	585.64	368.0	−21.0	441.00
4	376.0	391.1	−15.1	228.01	397.1	−21.1	445.21	394.3	−18.3	334.89
5	407.9	409.2	− 1.3	1.69	415.5	− 7.6	57.76	413.5	− 5.6	31.36
6	428.2	423.4	+ 4.8	23.04	429.3	− 1.1	1.21	428.1	+ .1	.01
7	447.1	434.7	+12.4	153.76	439.8	+ 7.3	53.29	439.3	+ 7.8	60.84
8	457.2	443.8	+13.4	179.56	447.8	+ 9.4	88.36	448.2	+ 9.0	81.00
9	464.2	451.2	+13.0	169.00	454.0	+10.2	104.04	455.0	+ 9.2	84.64
10	466.3	457.7	+ 8.6	73.96	458.6	+ 7.7	59.29	460.3	+ 6.0	36.00
11	468.0	461.6	+ 6.4	40.96	462.0	+ 6.0	36.00	464.2	+ 3.8	14.44
12	466.6	465.1	+ 1.5	2.25	464.4	+ 2.2	4.84	467.0	− .4	.16
13	466.6	467.5	− .9	.81	465.8	+ .8	.64	468.8	− 2.2	4.84
14	467.0	469.0	− 2.0	4.00	466.6	+ .4	.16	469.7	− 2.7	7.29
15	466.6	469.6	− 3.0	9.00	466.6	0	0	469.7	− 3.1	9.61
16	464.6	469.4	− 4.8	23.04	466.0	− 1.4	1.96	469.0	− 4.4	19.36
17	462.5	468.5	− 6.0	36.00	464.9	− 2.4	5.76	467.7	− 5.2	27.04
18	460.7	466.9	− 6.2	38.44	463.2	− 2.5	6.25	465.7	− 5.0	25.00
19	460.2	464.5	− 4.3	18.49	461.1	− .9	.81	463.0	− 2.8	7.84
20	455.2	461.5	− 6.3	39.69	458.6	− 3.4	11.56	459.9	− 4.7	22.09
21	453.7	457.9	− 4.2	17.64	455.7	− 2.0	4.00	456.2	− 2.5	6.25
22	449.1	453.7	− 4.6	21.16	452.4	− 3.3	10.89	452.0	− 2.9	8.41
23	444.0	448.9	− 4.9	24.01	448.8	− 4.8	23.04	447.3	− 3.3	10.89
24	443.2	443.5	− .3	.09	444.9	− 1.7	2.89	443.1	+ 1.1	1.21
25	448.7	437.5	+11.2	125.44	440.7	+ 8.0	64.00	436.4	+12.3	151.29
26	440.0	431.0	+ 9.0	81.00	436.2	+ 3.8	14.44	430.4	+ 9.6	92.16
Total	.....	.....	198.1	2,289.55	.....	177.9	2,743.33	.....	187.0	2,641.64

Least squares: Root mean-square error= 9.4; mean error= 7.6.
Moments, equal intervals: Root mean-square error= 10.3; mean error= 6.8.
Moments, unequal intervals: Root mean-square error= 10.1; mean error= 7.2.

In Table V are given the values of the *j*'s and certain other constants involving only *l* for values of *l* from 3 to 40. This range will include practically all cases likely to occur in ordinary statistical work.

The author wishes to acknowledge his indebtedness to Dr. Pearl for his aid and suggestions throughout the work.

TABLE V.—*Values of certain constants involving only l for values from 3 to 40*

	j_1	j_2	j_3	$l^{2.5}+.5l^{1.5}$	$\frac{6}{l^3}$	j_4	j_5	j_6	j_7	j_8	$\frac{30}{l^6}$
3	1.6701993	0.250410	0.250623	19.5	0.2222222						
4	.5832445	.205866	.318198	29.5	.0937500	0.5175122	327	192.5	0.0502309	7.583333	0.2929969
5	.2636105	.175228	.410207	41.5	.0480000	.1741179	405	315.0	.0365500	11.083333	.0096000
6	.1383150	.152774	.471447	55.5	.0277778	.0726607	627	479.5	.0278930	15.250000	.0038580
7	.0810052	.1355697	.524723	71.5	.0174027	.0350722	813	692.0	.0220353	20.083333	.0017850
8	.0512108	.121936	.572038	89.5	.0117188	.0187981	1,023	958.5	.0178776	25.583333	.0009553
9	.0342957	.110857	.614471	109.5	.0082305	.0109020	1,257	1,285.0	.0148139	31.750000	.0005805
10	.0240239	.101667	.653006	131.5	.0060000	.0067235	1,515	1,677.5	.0124876	38.583333	.0003900
11	.0174459	.093915	.688300	155.5	.0045079	.0043556	1,797	2,142.0	.0106774	46.083333	.0002868
12	.0130483	.087284	.720863	181.5	.0034722	.0029376	2,103	2,684.5	.0093398	54.250000	.0002056
13	.0100021	.081545	.751092	209.5	.0027310	.0020488	2,433*	3,311.0	.0080782	63.083333	.0001580799
14	.0078282	.076527	.779302	239.5	.0021866	.0014700	2,787	4,027.5	.0071255	72.583333	.0000055780
15	.0062369	.072102	.805749	271.5	.0017778	.0010807	3,165	4,840.0	.0063342	82.750000	.0000039506
16	.0050404	.068168	.830643	305.5	.0014648	.0008139	3,567	5,754.5	.0056694	93.583333	.0000028610
17	.0041386	.064649	.854157	341.5	.0012212	.0006203	3,993	6,777.5	.0051053	105.083333	.0000021129
18	.0034347	.061480	.876438	379.5	.0010288	.0004821	4,443	7,913.5	.0046234	117.250000	.0000015877
19	.0028809	.058612	.897610	419.5	.0008747 6	.0003809	4,917	9,170.0	.0042056	130.083333	.0000012116
20	.0024392	.056003	.917779	461.5	.0007500	.0003053	5,415	10,552.5	.0038435	143.583333	.0000093750
21	.0020829	.053620	.937038	505.5	.0006478 8	.0002452	5,937	12,067.0	.0035266	157.750000	.0000073456
22	.0017923	.051434	.955401	551.5	.0005634 9	.0002001	6,483	13,719.5	.0032478	172.583333	.0000058211
23	.0015531	.049421	.973123	599.5	.0004931 4	.0001649	7,053	15,516.0	.0030012	188.083333	.0000046610
24	.0013544	.047562	.990084	649.5	.0004340 3	.0001371 1	7,647	17,462.5	.0027818	204.250000	.0000037676
25	.0011880	.045840	1.006397	701.5	.0003840 0	.0001148 7	8,265	19,565.0	.0025859	221.083333	.0000030720
26	.0010476	.044239	1.022111	755.5	.0003413 7	.0000969 40	8,907	21,829.5	.0024102	238.583333	.0000025259
27	.0009284 0	.042748	1.037267	811.5	.0003048 3	.0000823 59	9,573	24,262.0	.0022520	256.750000	.0000020908
28	.0008265 2	.041355	1.051905	869.5	.0002733 2	.0000704 06	10,263	26,868.5	.0021090	275.583333	.0000017431
29	.0007385 5	.040050	1.066059	929.5	.0002460 1	.0000605 35	10,977	29,655.0	.0019793	295.083333	.0000014626
30	.0006617	.038827	1.079761	991.5	.0002222 2	.0000523 26	11,715	32,627.5	.0018613	315.250000	.0000012346
31	.0005953	.037677	1.093038	1,055.5	.0002014 0	.0000454 57	12,477	35,792.0	.0017537	336.083333	.0000010479
32	.0005401 6	.036593	1.105916	1,121.5	.0001831 1	.0000396 73	13,263	39,154.5	.0016552	357.583333	.0000089407
33	.0004898 7	.035571	1.118418	1,189.5	.0001669 6	.0000347 77	14,073	42,721.0	.0015649	379.750000	.0000076057
34	.0004456 0	.034605	1.130567	1,259.5	.0001526 6	.0000306 10	14,907	46,497.5	.0014818	402.583333	.0000066028
35	.0004060 50	.033690	1.142380	1,331.5	.0001399 4	.0000270 46	15,765	50,490.0	.0014052	426.083333	.0000057119
36	.0003718 1	.032823	1.153878	1,405.5	.0001286 0	.0000239 84	16,647	54,704.5	.0013344	450.250000	.0000049615
37	.0003409 5	.032000	1.165075	1,481.5	.0001184 5	.0000213 41	17,553	59,147.0	.0012689	475.083333	.0000043263
38	.0003134 0	.031218	1.175988	1,559.5	.0001093 5	.0000190 51	18,483	63,823.5	.0012081	500.583333	.0000037862
39	.0002887 2	.030473	1.186631	1,639.5	.0001011 5	.0000170 59	19,437	68,740.0	.0011517	526.750000	.0000033251
40	.0002665 6	.029763	1.197017	1,721.5	.0000937 5	.0000153 20	20,415	73,902.5	.0010991	553.583333	.0000029297

LITERATURE CITED

(1) CURTIS, M. R.
1914. A biometrical study of egg production in the domestic fowl. IV. Factors influencing the size, shape, and physical constitution of eggs. *In* Arch. Entwicklungsmech. Organ., Bd. 39, Heft 2/3, p. 217-327, pl. 6-10.

(2) DONALDSON, H. H.
1908. A comparison of the albino rat with man in respect to the growth of the brain and of the spinal cord. *In* Jour. Compar. Neurol. and Psych., v. 18, no. 4, p. 345-389.

(3) ———
1909. On the relation of the body length to the body weight and to the weight of the brain and of the spinal cord in the albino rat (Mus norvegicus var. albus). *In* Jour. Compar. Neurol. and Psych., v. 19, no. 2, p. 155-167.

(4) ———
1910. On the percentage of water in the brain and in the spinal cord of the albino rat. *In* Jour. Compar. Neurol. and Psych., v. 20, no. 3, p. 119-144.

(5) ———
1911. On the regular seasonal changes in the relative weight of the central nervous system of the leopard frog. *In* Jour. Morph., v. 22, p. 663-694.

(6) ——— and HATAI, SHINKISHI.
1911. A comparison of the Norway rat with the albino rat in respect to body length, brain weight, spinal cord weight, and the percentage of water in both the brain and the spinal cord. *In* Jour. Compar. Neurol. and Psych., v. 21, p. 417-458.

(7) ELDERTON, W. P.
1907. Frequency-curves and correlation. 172 p. London.

(8) HATAI, SHINKISHI.
1909. Note on the formulas used for calculating the weight of the brain in the albino rats. *In* Jour. Compar. Neurol. and Psych., v. 19, no. 2, 169-173.

(9) ———
1911. A formula for determining the total length of the leopard frog (R. pipiens) for a given body weight. *In* Anat. Rec., v. 5, no. 6, p. 309-312.

(10) ———
1911. An interpretation of growth curves from a dynamical standpoint. *In* Anat. Rec., v. 5, no. 8, p. 373-382.

(11) HOLSTEIN-FRIESIAN ASSOCIATION OF AMERICA.
1913. The advanced registry year book. v. 24, 1004 p.

(12) HOLTSMARK, G.
1904. [On the relation of milk yield and feed consumption.] *In* Arch. Math. og Naturvidensk. v. 26, no. 2. (Cited from Exp. Sta. Rec., v. 16, p. 591.)

(13) LEWENZ, M. A., and PEARSON, KARL.
1904. On the measurement of internal capacity from cranial circumference. *In* Biometrika, v. 3, pt. 4, p. 366-397, pl. 2-3.

(14) PEARL, RAYMOND.
1909. Studies on the physiology of reproduction in the domestic fowl. I. Regulation in the morphogenetic activity of the oviduct. *In* Jour Exp. Zool., v. 6, no. 3, p. 339-359, 2 fig.

(15) ———
1914. On the law relating milk flow to age in dairy cattle. *In* Proc. Soc. Exp. Biol. and Med., v. 12, no. 1, p. 18-19.

(16) ——— and MCPHETERS, LOTTIE E.
1911. A note on certain biometrical computations. *In* Amer. Nat., v. 45, no. 540, p. 756-760.

(17) — — with the assistance of PEPPER, OLIVE M., and HAGLE, FLORENCE J.
1907. Variation and differentiation in Ceratophyllum. 136 p. Washington, D. C. (Carnegie Inst. Washington, Pub. 58.)

(18) PEARSON, KARL.
1895. Mathematical contribution to the theory of evolution. II. Skew variations in homogeneous material. *In* Phil. Trans. Roy. Soc. London, s. A., v. 186, p. 343-414, 10 pl.

(19) ———
1899. On certain properties of the hypergeometrical series, and on the fitting of such series to observation polygons in the theory of chance. *In* Phil. Mag. and Jour. Sci., s. 5, v. 47, no. 285, p. 236-246.

(20) PEARSON, KARL.
1901. Mathematical contributions to the theory of evolution. X. Supplement to a memoir on skew variation. *In* Phil. Trans. Roy. Soc. London, s. A., v. 197, p. 443–459.

(21) ———
1902. On the systematic fitting of curves to observations and measurements. II. *In* Biometrika, v. 2, pt. 1, p. 1–23, 10 fig.

(22) ———
1904. On the laws of inheritance in man. II. On the inheritance of mental and moral characters in man, and its comparison with the inheritance of the physical characters. *In* Biometrika, v. 3, pt. 2/3, p. 131–190.

(23) ———
1907. On the influence of past experience on future expectation. *In* Phil. Mag. and Jour. Sci., s. 6, v. 13, no. 75, p. 365–378.

75012°—15——6

ORGANIC PHOSPHORIC ACID OF RICE

By ALICE R. THOMPSON,
Assistant Chemist, Hawaii Agricultural Experiment Station

That phosphoric acid occurs in organic combinations with inosite in the seeds of many plants has been shown by several investigators. Posternak (9),[1] Patten and Hart (8), Hart and Andrews (6), Hart and Tottingham (7), Anderson (1, 2, 3, 5), Rather (10, 11), and others have isolated this organic substance from pumpkin seed, beans, wheat, corn, oats, and cotton seed. Although they have been unable to obtain it by synthesis and still disagree as to the formula and composition of the acid and its salts, it is generally known as phytin or phytic acid. Suzuki et al. (12, 13) and Anderson believe phytin to be a hexaphosphoric acid ester of inosite, and Anderson has shown that the organic phosphoric acid in wheat bran differs materially from the acid he has obtained from a number of seeds.

Phytin is completely hydrolyzed into free phosphoric acid and inosite only with difficulty, as Anderson (4) showed by boiling phytin with concentrated nitric acid for several hours.

In the previous determination of phosphoric acid in foliage and grain of rice (*Oryza sativa*) at the Hawaii Experiment Station (14) several methods were used in oxidizing the organic matter. On boiling the grain with a mixture of nitric and hydrochloric acids (aqua regia) the author noticed that, although the solution soon became colorless, giving the appearance of complete oxidation of organic matter, if boiled to dryness a charred mass remained in the flask. Determinations of phosphoric acid in the solution (not boiled to dryness) in the case of the rice grain showed about one-third of the total phosphoric acid as found by the Neumann method. The determination of phosphoric acid in the foliage, on the other hand, by either method was about the same.

It was thought that the reason for this resistance to the action of aqua regia is probably the fact that phosphoric acid occurs in the rice grain as phytin and is therefore not completely hydrolyzed. It was decided, therefore, to give some study to the organic phosphoric acid of rice.

Suzuki et al. (12, 13) obtained an impure salt of phytic acid from rice by extracting the rice bran with a 0.2 per cent hydrochloric-acid solution and precipitating with alcohol. As Anderson (1, 2, 3, 4, 5) has shown that phytin so prepared would contain the inorganic phosphoric acid of the seed, as well as other impurities, the phosphorus content as shown by the resulting analysis is not that of pure phytin.

It is therefore of interest to obtain the pure salt of phytic acid from rice. In following the methods of Anderson (1, 2, 3, 4, 5) of purifying the acid by repeated solution in hydrochloric acid and precipitation with barium hydroxid, the author hoped to isolate the pure tribarium salt, but the ease of partial hydrolysis of the substance and the difficulty of eliminating all impurities which may be present, such as other phos-

[1] Reference made by number to "Literature cited," p. 430.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. III, No. 5
Feb. 15, 1915
B 4

phoric esters of inosite, stand in the way of obtaining the pure substance. Special attention was paid to methods for the determination of the barium and phosphoric acid in the salt, and observations of some interest were made.

The total phosphorus was determined in samples of rice bran and unpolished and polished rice. The following determinations were duplicated to within 0.02 per cent: Phosphorus in rice bran, 2.291 per cent; in unpolished rice, 0.321 per cent; in polished rice, 0.140 per cent.

It is apparent that the rice bran contains a comparatively high percentage of phosphorus. Phytin was determined in the bran by extracting 100 gm. of the sample with a 0.2 per cent hydrochloric-acid solution and precipitating with alcohol. The precipitate stood over night, when it was filtered, first washed with 50 per cent alcohol, then with ether, and was dried at 105° C. It weighed 8.22 gm., amounting, therefore, to 8.22 per cent of the bran. As phytin contains considerable organic phosphorus, it is apparent that rice bran contains much of its phosphorus in the organic form.

The writer was unable to obtain phytin from the polished rice. The 0.2 per cent hydrochloric-acid extract from several kilograms of finely ground polished rice yielded but a slight precipitate with alcohol or barium chlorid. With barium hydroxid added to alkalinity a considerable precipitate occurred which did not behave like phytin.

The phytin obtained from the unpolished rice was doubtless contained in the outer layer, which is removed in polishing.

Two preparations of barium phytate were made according to the method of Anderson; one from unpolished rice, the other from rice bran.

Six kg. of finely ground unpolished rice were treated with a 0.2 per cent hydrochloric-acid solution for several hours. This was filtered through cheesecloth and filter paper the same day and the filtrate precipitated with a barium-chlorid solution.

The precipitate was collected on a filter, washed with 50 per cent alcohol, dissolved in a 1.0 per cent hydrochloric-acid solution, which was then filtered and precipitated with barium hydroxid. It was reprecipitated three additional times with barium hydroxid, once with alcohol, again with barium hydroxid, and again three times with alcohol. The filtrate from the alcohol precipitate soon ceased to give an immediate precipitate with an ammonium-molybdic solution, showing the elimination of inorganic phosphoric acid from the precipitate. The precipitates formed by barium hydroxid were well washed with water.

Crystals were then obtained by adding barium hydroxid to the acid solution until a slight precipitate resulted and then filtering and allowing to stand a couple of days. The crystals thus formed were washed, recrystallized in the same way, and then crystallized from the acid solution by the addition of alcohol. It was found that on adding an equal volume of alcohol, according to Anderson, only an amorphous precipitate was obtained. It was only by adding just sufficient alcohol to make the solution turbid that crystallization took place.

Under the microscope the precipitate appeared to be composed entirely of crystals in the form of globules of needles with occasional needle-shaped crystals arranged in stellar form. These were probably the same substance.

The crystals were washed with alcohol and ether, dried in vacuum over sulphuric acid at laboratory temperature, and finally at 105° to 110° C.

over calcium chlorid without vacuum. The moisture was then determined in the salt at 120° to 130° C. and amounted to 0.76 per cent. The phytin from the bran was purified and obtained in the same way as that from unpolished rice, but the barium salt was dried in vacuum over calcium chlorid at 105° C. The moisture determined in vacuum at 105° and at 120° C. was 1.33 per cent in both cases. The crystals resembled those of the first preparation.

The salts thus obtained were practically free from chlorids and inorganic phosphates. Nitrogen was also absent. All the material of the first preparation was used in making repeated determinations of barium, phosphorus, carbon, and hydrogen, but the phytin obtained from the bran was analyzed also for ash constituents other than barium. In 0.60 gm. of this material an unweighable trace of calcium was found, but no iron, manganese, magnesium, or potash. The residue on precipitating out the barium and igniting the phytic acid thus left amounted to a few milligrams and was composed mostly of unvolatilized phosphoric acid. No nitrogen was found in the salt.

The barium in the salts was determined by various methods. A precipitate of barium sulphate was obtained in the determination of phosphorus by the Neumann method. After boiling the salts for three to four hours with sulphuric acid to which ammonium nitrate was added at intervals, 200 c. c. of water were added to the solution, and the precipitate of barium sulphate resulting from this dilution was boiled and allowed to stand overnight on the water bath. The precipitate was collected on a filter and was washed well, dried, and ignited. This precipitate was not pure barium sulphate, but contained a large amount of silica, aluminum, etc., dissolved from the Kjeldahl flask by the concentrated solution of very hot ammonium nitrate in sulphuric acid. On heating the weighed precipitate with hydrofluoric and sulphuric acids, silica was eliminated and the weight of the residue considerably reduced. In one case 0.6853 gm. of precipitate lost 0.0111 gm. by this treatment. A pure barium sulphate was also obtained by fusing the residue with sodium carbonate. The fusion was digested in boiling water, then filtered and washed. The filtrate was tested for barium and the insoluble residue dissolved in a few drops of dilute hydrochloric acid, washed through the filter paper, and the barium precipitated in boiling solution by the slow addition of 0.4 N sulphuric acid.

A number of determinations were thus made in which the correction by fusion caused a decrease in calculated barium from 1 to 2 per cent. The results from three samples given in Table I are taken from the analysis of the phytin from bran (not calculated to moisture-free basis).

TABLE I.—*Percentage of barium in barium phytate oxidized by the Neumann method*

Sample No.	Barium.	
	Uncorrected.	Corrected by fusion.
	Per cent.	*Per cent.*
1	38.293	37.291
2	38.256	37.358
3	38.191	37.305

Two of the corrected residues were treated with hydrofluoric acid, but no decrease in weight resulted.

Barium was also determined by dissolving the barium phytate in about 200 c. c. of water with a few cubic centimeters of dilute hydrochloric acid. Twenty c.c. of 0.4 *N* sulphuric acid were added to the boiling solution, the whole was boiled about half an hour, and then set aside on a hot-water bath for several hours. The precipitate, after standing overnight, was filtered, washed, and ignited. On weighing, then fusing and reprecipitating the barium, as was done above, a slight increase in weight was observed. The filtrates from the barium precipitates after fusion were united, evaporated to small bulk, acidified with nitric acid, and ammonium molybdate added. A yellow precipitate was found after standing, and the phosphoric acid then obtained was weighed as magnesium pyrophosphate. By fusing the impure residues amounting to from 0.2754 to 0.3511 gm., the magnesium pyrophosphate obtained was found to be 0.0181, 0.0092, 0.0095, and 0.0074 gm.

The weight of the residues of barium sulphate before and after correction for phosphate is given in Table II.

TABLE II.—*Quantity of barium sulphate* [a] *and magnesium pyrophosphate precipitated from a solution of barium phytate*

Samples No.	Uncorrected.	Fused and corrected.	Phosphoric acid as magnesium pyrophosphate.
	Gm.	*Gm.*	*Gm.*
1	0.2754	0.2785	0.0181
2	.3117	.3128	.0092
3	.4672	.4703	.....

[a] Not calculated to water-free basis.

The filtrate containing the phytic acid from which the barium was precipitated by the foregoing method still contained a few tenths of a per cent of barium. On evaporating to small bulk or igniting, a small precipitate of barium sulphate was obtained. It would appear that phytic acid has some solvent action on barium sulphate.

The fact that phosphoric acid was precipitated along with the barium sulphate by very dilute sulphuric acid suggests that the composition of phytic acid as determined by Anderson may have been affected. The phosphorus, if carried down with the barium sulphate, would cause a low phosphorus content in the remaining solution.

It was attempted to determine barium by first igniting the salt, but a white ash could not be obtained, and the residue was extremely difficult to dissolve after ignition. Phosphoric acid was best determined by the Neumann method, filtering off the barium sulphate formed on dilution. The results were about 0.1 per cent lower when determined by precipitating the barium with dilute sulphuric acid and evaporating the phytic acid thus obtained with magnesium nitrate and igniting and adding the phosphorus anhydrid found in the fused barium residue.

Carbon and hydrogen were determined by the regular combustion method, passing oxygen through the apparatus during the burning. In each case the black ash remaining was ground up with potassium dichromate and reburned.

The analyses of the barium phytate are given in Table III and are calculated to the water-free basis. The barium and phosphorus content is lower than that reported by Anderson for tribarium-inosite-hexaphosphate. Whether the barium phytate obtained was composed of a single salt of inosite is not absolutely certain.

TABLE III.—*Analyses of barium phytate calculated to the water-free basis*

Constituent.	Source of barium phytate			
	Unpolished rice.		Rice bran.	
	Sample No. 1.	Sample No. 2.	Sample No. 1.	Sample No. 2.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
C	6. 63	6. 97	6. 62	6. 51
H	1. 75	1. 84	1. 82	1. 87
P	16. 43	16. 38	16. 06	16. 05
Ba	36. 93	36. 84	37. 79	37. 84

UNPOLISHED RICE [a]

Sample No.	Quantity used.	H_2O	CO_2	$Mg_2P_2O_7$	$BaSO_4$
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	0. 3729	0. 0607	0. 0900	.....	.....
2	. 3551	. 0605	. 0901	.....	.....
1	. 4752	.....	.....	0. 2779	.....
2	. 2365	.....	.....	. 1379	.....
1	. 4514	.....	.....	.....	0. 2811
2	. 5042	.....	.....	.....	. 3132

RICE BRAN [b]

Sample No.	Quantity used.	H_2O	CO_2	$Mg_2P_2O_7$	$BaSO_4$
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	0. 2559	0. 0445	0. 0613	.....	.....
2	. 3271	. 0583	. 0771	.....	.....
1	. 5514	.....	.....	0. 3137	.....
2	. 4765	.....	.....	. 2709	.....
1	. 5514	.....	.....	.....	0. 3496
2	. 6730	.....	.....	.....	. 4251

[a] 0.5107 gm. lost 0.0510 gm. H_2O = 9.75 per cent of moisture.
[b] 0.9717 gm. lost 0.0129 gm. H_2O = 1.33 per cent of moisture.

Inosite was prepared from the barium phytate of rice bran by heating in sealed tubes to 150° C. about 2 gm. of the salt with 20 c. c. of 30 per cent sulphuric acid for five hours.

The sulphuric acid was precipitated with barium hydroxid, the excess of barium removed by carbon dioxid, and the filtrate evaporated to dryness. The residue was extracted with hot water and filtered. The inosite was precipitated by ether and alcohol and recrystallized three times as minute needles. These gave the Scherer reaction and melted at 223° C., uncorrected.

Thanks are due Dr. W. P. Kelley, of the Hawaii Experiment Station, who suggested this work on phytin in rice and gave helpful advice throughout.

LITERATURE CITED

(1) ANDERSON, R. J.
1912. The organic-phosphoric acid compound of wheat bran. N. Y. State Agr. Exp. Sta. Tech. Bul. 22, 16 p.

(2) ———
1912. The organic phosphoric acid of cottonseed meal. N. Y. State Agr. Exp. Sta. Tech. Bul. 25, 12 p.

(3) ———
1912. Phytin and phosphoric acid esters of inosite. N. Y. State Agr. Exp. Sta. Tech. Bul. 19, 17 p.

(4) ———
1912. Phytin and pyrophosphoric acid esters of inosite.—II. N. Y. State Agr. Exp. Sta. Tech. Bul. 21, 16 p.

(5) ———
1914. A contribution to the chemistry of phytin. N. Y. State Agr. Exp. Sta. Tech. Bul. 32, 44 p.

(6) HART, E. B., and ANDREWS, W. H.
1903. The status of phosphorus in certain food materials and animal by-products, with special reference to the presence of inorganic forms. *In* Amer. Chem. Jour., v. 30, no. 6, p. 470–485.

(7) ——— and TOTTINGHAM, W. E.
1910. The nature of the acid-soluble phosphorus compounds of some important feeding materials. Wis. Agr. Exp. Sta. Research Bul. 9, p. 95–106.

(8) PATTEN, A. J., and HART, E. B.
1904. The nature of the principal phosphorus compounds in wheat bran. *In* Amer. Chem. Jour., v. 31, no. 5, p. 564–572.

(9) POSTERNAK, S.
1900. Contribution a l'étude chimique de l'assimilation chlorophyllienne. Sur le premier produit d'organization de l'acide phosphorique dans les plantes à chlorophylle avec quelques remarques sur le rôle physiologique de l'inosite. *In* Rev. Gén. Bot., t. 12, no. 133, p. 5–24.

(10) RATHER, J. B.
1912. The forms of phosphorus in cottonseed meal. Texas Agr. Exp. Sta. Bul. 146, 16 p.

(11) ———
1913. The phosphorus compounds of cottonseed meal and wheat bran. Texas Agr. Exp. Sta. Bul. 156, 18 p.

(12) SUZUKI, UMETARO, and YOSHIMURA, K.
1907. Ueber die Verbreitung von "anhydro-oxy-methylen-diphosphorsauren Salzen" oder "Phytin" in Pflanzen. *In* Bul. Col. Agr. Tokyo Imp. Univ. Japan, v. 7, no. 4, p. 495–502.

(13) ———, ———, and TAKAISHI, M.
1907. Ueber ein Enzym "Phytase," das "anhydro-oxy-methylen diphosphorsäure" spaltet. *In* Bul. Col. Agr. Tokyo Imp. Univ. Japan, v. 7, no. 4, p. 503–512.

(14) THOMPSON, ALICE R.
1913. The determination of sulphur and chlorin in the rice plant. *In* Hawaii Agr. Exp. Sta. Ann. Rpt., 1912, p. 64–73.

TWO CLOVER APHIDS[1]

By EDITH M. PATCH,
Entomologist, Maine Agricultural Experiment Station

It recently came to my attention that two distinct species of clover aphids are rather generally confused in collections under the name "*Aphis bakeri.*" As the range of both species extends nearly, if not quite, across the continent, it is a matter of more than local interest that this confusion should be straightened out. Specimens have been received from Messrs. J. J. Davis, A. Maxson, and H. F. Wilson, and I am indebted to Prof. C. P. Gillette for reading the manuscript and for the determination as to which species should properly be known as *Aphis bakeri.*

I have been unable to secure a type specimen of *Aphis brevis* for examination, although through the kindness of Dr. L. O. Howard, Chief of the Bureau of Entomology, and Mr. H. Hayward, Director of the Delaware Experiment Station, a thorough search was made both at Washington and at Newark. I feel confident, however, that Prof. Sanderson's careful description and figures[2] are sufficient to enable us to refer the long-beaked clover aphid to that species with safety.

Aphis brevis Sanderson (Long-beaked clover aphid).

In the vicinity of Orono, Me., the leaves of the hawthorn (*Crataegus* spp.) in June are commonly twisted into dark-purple swollen curls and are inhabited by an aphid the fall migrants of which were described by Prof. Sanderson as *Aphis brevis.*[2] This insect takes flight from hawthorn during June and early July and returns late in the season before producing the sexual generation. I have taken the fall migrants on cultivated plum (*Prunus* spp.), but as yet have made no spring collections from that host. In June and July, 1906, I collected apparently the same species from the twigs and terminal leaf curls of the Japan quince (*Cydonia japonica*).

Not being able to find characters to separate these collections from certain specimens labeled "*Aphis bakeri*" received from the Middle West, I undertook some transfer tests during the summer of 1912, and found that my *Aphis brevis* accepted both alsike and other clover (*Trifolium* spp.). Migrants placed on alsike and white clover produced nymphs that fed with apparent satisfaction on the test plants. The potted white clover was, however, more easily managed in the laboratory, so it was selected for the main rearings. The transfer was made on June 14. The migrants fed on the clover, and their abdomens became distended. At this time the head, thorax, and cornicles were black, and abdomens olive green, with distinct black lateral dots. By June 21 their abundant progeny were established on both stem and runner. The nymphs at first were pale and pellucid, with rosy head and prothorax. By June 24 this generation had matured, but did not begin to reproduce for a day or two. By June

[1] Papers from the Maine Agricultural Experiment Station: Entomology No. 76.
[2] Sanderson, E. Dwight. Report of the entomologist. *In* Del. Agr. Exp. Sta. 13th Ann. Rpt. [1900] 101, p. 157-158. 1902.

27 these adults had lost the rosy hue they had as nymphs and had become creamy white or grayish white with black antennæ, dusky legs, and deep rusty spots at base of cornicles, but with no rusty line connecting them.

On August 5, 1912, my attention was called to infested sweet-pea (*Lathyrus odoratus*) vines, which had vigorous colonies of red aphids on the stems at the surface of the ground extending for an inch up the plants. These proved to be the long-beaked clover aphids, and the source of the infestation doubtless was the hawthorn tree a few rods distant which had been heavily attacked by the spring generations of this species earlier in the season.

The spring forms on the hawthorn include two types of apterous females. One, possibly the stem mother, has the head, prothorax, and thorax soft coral pink. Joints I, II, and III of the antenna are coral pink or pellucid, while the other joints are black. The abdomen is olive green mottled with brown and pink, with a slight bloom only. The very short cornicle is light pellucid with the merest dusky tip, and the very short cauda is dark brown.

The apterous female of the second (or third?) generation has the head, prothorax, and thorax crimson, overcast with a slight bloom. Joints I, II, and III of the antenna are pellucid, while IV, V, and VI are black. The abdomen is crimson mottled with olive green and

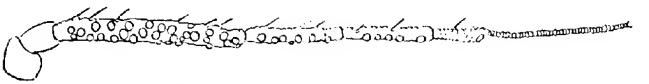


FIG. 1.—*Aphis brevis*: Antenna of fall alate female collected from hawthorn.

covered with slight bloom. There is a pale olive space about the base of the short cornicles, which are light olive green.

The spring migrant before flight has the dorsal surface of the head shining black, the ventral reddish, with a black beak and the antennæ black; the prothorax is black with red membrane; the dorsal lobes of the thorax are shining black, the breast reddish black; the dorsum of the abdomen is red in form of a heavy cross, the part about the cornicles being pale olive green; the venter is red. These same migrants after feeding on clover juice lose with age their reddish cast, the abdomens then becoming olive green.

The nymph, which is to become the spring migrant, in the pre-imago stage has its head and thorax coral pink, its abdomen red and more or less mottled, with a pale olive green space about the cornicle extending over several segments.

The fall alate form does not differ essentially from the spring migrant. The sensoria of the antenna (fig. 1) are distributed over joints III, IV, and V, in practically the same numbers as the spring migrant, although some individuals of the spring form have fewer to none on joint V. V and VI are nearly subequal, and they are both sometimes longer than those shown in figure 1. Joint V is sometimes a little shorter than IV.

Joints IV, V, and VI in the male are relatively longer than those in the alate female and there are frequently more sensoria on IV and V, as well as sometimes from one to several on basal part of joint VI (fig. 2).

The lateral tubercles of the prothorax and abdomen are distinct and blunt in both the alate and the apterous generations.

The beak in the apterous forms ordinarily reaches easily to the second coxa, and in the alate forms well beyond the second coxa, sometimes reaching the third. The most distinctive character of the wing is the short, broad stigma with a blunt distal end.

Aphis bakeri Cowen (Short-beaked clover aphid).

About the middle of August, 1914, large numbers of an aphid from *Trifolium pratense* were taken by Mr. George Newman at Orono, Maine. This species was distinct from the one just discussed, and yet I found that it was commonly listed in collections as *Aphis bakeri*. In the

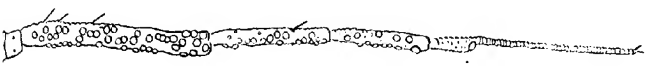


FIG. 2.—*Aphis brevis*: Antenna of alate male.

original description of *Aphis cephalicola* Cowen,[1] a synonym of *A. bakeri*, according to Gillette and Taylor,[2] the specifications "Third joint of antennæ tuberculate, with numerous irregular sensoria, fourth with few irregular sensoria," and "Beak hardly reaching second coxa" at once applied to *A. bakeri* of this paper and distinctly did not apply to *A. brevis*. *Aphis bakeri* is found also upon shepherd's-purse (*Capsella bursa-pastoris*) in the fall and early spring, but whether there is a migration between shepherd's-purse and clover I do not know. Mr. Wilson lent me specimens of this aphid collected from the hawthorn in Oregon. It occurs on apple (*Malus* spp.) in Colorado.[2] I have made a single collection of a fall migrant on hawthorn at Orono on October 1, 1914.

The habitat of the short-beaked clover aphid on clover seemed to be the ventral side of the leaf and the stem near the ground. The colonies

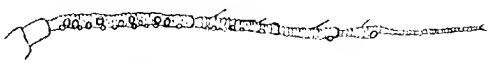


FIG. 3.—*Aphis bakeri*: Antenna of alate female collected from clover.

were frequently covered by "ant sheds," as well as sometimes extending for a short distance underground.

This species is smaller, more slender and graceful than the long-beaked clover aphid. Joint V of the antenna is noticeably shorter than IV and is without sensoria, except the usual distal one, in the summer winged viviparous female (fig. 3.) The stigma is rather narrow and the distal end acute. The beak hardly reaches the second coxa and frequently falls considerably short of it. The prothoracic and abdominal lateral tubercles are prominent, but very slender. Both species have the cornicles and cauda very short.

[1] Cowen, J. H. [Aphididæ.] *In* Gillette, C. P., and Baker, C. F. A preliminary list of the hemiptera of Colorado. Colo. Agr. Exp. Sta. Bul. 31 (Tech. Ser. 1), p. 118. 1895.

[2] Gillette, C. P., and Taylor, E. P. A few orchard plant lice. Colo. Agr. Exp. Sta. Bul. 133, 47 p., 1 fig., 4 pl. 1908.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

25 CENTS PER COPY

SUBSCRIPTION PER YEAR, 12 NUMBERS, $2.50.